AIRBORNE BACTERIA FROM LIQUID WASTE TREATMENT UNITS

By

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AIRBORNE BACTERIA FROM LIQUID WASTE

TREATMENT UNITS

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CHAPTER I

INTRODUCTION

Waste water treatment plants were originally built away from residential and commercial properties of a community because of the air pollution, water pollution, aesthetic conditions, and psychological factors. During the past few years the population explosion of many cities has caused the areas surrounding treatment plants to be engulfed. Problems have resulted in overloads for old treatment plants, and property disputes for new locations. This has caused more rigorous design and operation of treatment plants, but many problems still exist with plant operation, property value, public opinion, and public safety.

While considering bacterial infection, the major factors are: infective dosage, means of transportation, host that is susceptible to the bacteria, and a portal of entry (1). A treatment plant and the conditions surrounding this area might supply all of the necessary factors. Drying beds, trickling filters, and aeration tanks could place an infective dosage in the air as dust particles, aerosol droplets, and droplet nuclei. The droplets allow bacteria to remain viable and be carried great distances

by prevailing winds. The people living close to these areas undoubtedly provide a susceptible host and a portal of entry when they inhale this air. Thus, the necessary factors influencing infection are available.

This research was undertaken to determine if airborne bacteria were being emitted from wastwater treatment units, such as aeration tanks and trickling filters. This entailed the collection of a representative sample of aerosols produced, the incubation of the bacteria entrapped, and some means of classification.

CHAPTER II

LITERATURE REVIEW

The early theories of disease transmission were based on miasmic concepts. This type of theory existed as far back as 1600 B.C., when an Egyptian scholar prepared a medical treatise indicating that sickness was due to "winds" and the gods of the winds, seasons and sickness were associated (2). The Greeks believed in natural causes for illness upon the people, and about 400 B.C. Hippocrates taught that "airs, waters, and places" influenced the health of the population (3). The theory of the air transporting disease was believed through the Middle Ages. During the seventeenth century, Leeuwenhoek developed and made simple microscopes. Using these microscopes he observed small animalcules, and reported his microscopical discoveries to the Royal Society (1). The microbial world was forgotten until the nineteenth century, when improvements of the microscope advanced microscopical research. The developments brought concern over spontaneous generation and the presence of microorganisms.

Studies conducted during the nineteenth century by Pasteur and others showed the theory of germs and the cellu-

lar structure of living matter caused contamination and diseases (4). Pasteur conducted studies proving that sterile conditions would exist until microorganisms were inoculated. This convinced his French contemporaries that spontaneous generation was not the cause of disease (4). Pasteur showed that these microorganisms could be carried in the air. He also showed that the air contained varying amounts of organisms by exposing twenty sterile infusions at different locations, and observing the number of flasks showing growth at each location (4). Tyndall, a supporter of Pasteur, continued the research of airborne bacteria and discovered the ability of bacteria to exist as spores. This allowed others to explain the variance in their results, and it was concluded that airborne bacteria could cause contamination (5). The germs that contaminated and caused the diseases were not found in dust collected in the air, and this caused a doubt that air was the means of transportation. By the end of the century, most bacterial agents of common communicable disease had been isolated, and their means of transportation had been attributed to methods other than air (2).

Miquel conducted a long-term survey of the microbial content of the atmosphere by volumetric methods. He conducted this survey out-of-doors, in ships, and in Paris sewers. He indicated 800 to 900 bacteria per cubic meter in the sewers and noted the absence of pollens. These results were comparable with those reported from London

sewers by Garnelley and Haldane, but others were reporting the same amount in populated areas of these cities (3).

During the early part of 1930, there was a revival of interest in airborne infection and odors emitted from sewage plants. Fair and Wells (2) conducted a research project of bacteria emitted to the atmosphere by sewage disposal processes. They found that the bacterial count around sewage plants was high, and concluded that organisms liberated from these areas could cause respiratory diseases and skin infection. Several studies were conducted testing for pathogenic organisms in sewage waters, and relating the pathogen content to sewer gas and odors (6). It was found that odors and bacteria were related, but do not necessarily co-exist. The odors found in the sewers were caused from gases such as hydrogen sulfide without the presence of bacteria, and also that bacteria could be present in the air without any particular odors. The odors emitted from sewers were a combination of both chemicals present and bacterial content (7). The legal suit on water rights in Illinois (8) declared that while odors were a nuisance, they were not a menace to health unless actual damage could be shown.

The study of respiratory diseases aroused other concerns, such as the ability of particles to remain in the lungs. Brown and Cook (9) undertook a project to determine the influence of particle size upon the retention of particulate matter in the human lung. They concluded that the nasal chamber had one hundred per cent filtering efficiency

for particles ranging from five microns to ten microns, and relatively no detention of particles smaller than one micron. The maximum deposition of particles in the lungs was in the one micron range, and decreased both ways due to the large particles being trapped in the upper respiratory track and smaller particles being exhaled (9). Several studies were made to determine the exact relationship controlling the deposition and retention of airborne particles in the lungs. Different methods of determining the ability of the respiratory tract to collect airborne particles has been graphically summarized in Figure 1. This graph considers three areas of deposition, alveolar, tracheobronchial, and masopharyngeal. This was an attempt to convert all particles to unit density spheres, and show them by their aerodynamic size (10).

Wells (2) conducted a study of airborne contagion and air hygiene. He was interested in sanitary ventilation, and the prevention of spreading contagion through populated areas. He studied the biology parasites in droplet nuclei, evaporation and condensation of droplets, and their aerodynamic abilities. Through these studies he concluded that infection could be spread by airborne particles, and should be controlled.

Randall and Ledbetter (11) conducted a series of tests for airborne bacteria around aerobic waste treatment plants. Because of the wide variety of bacteria that may be emitted from these plants, their study was restricted to enteric

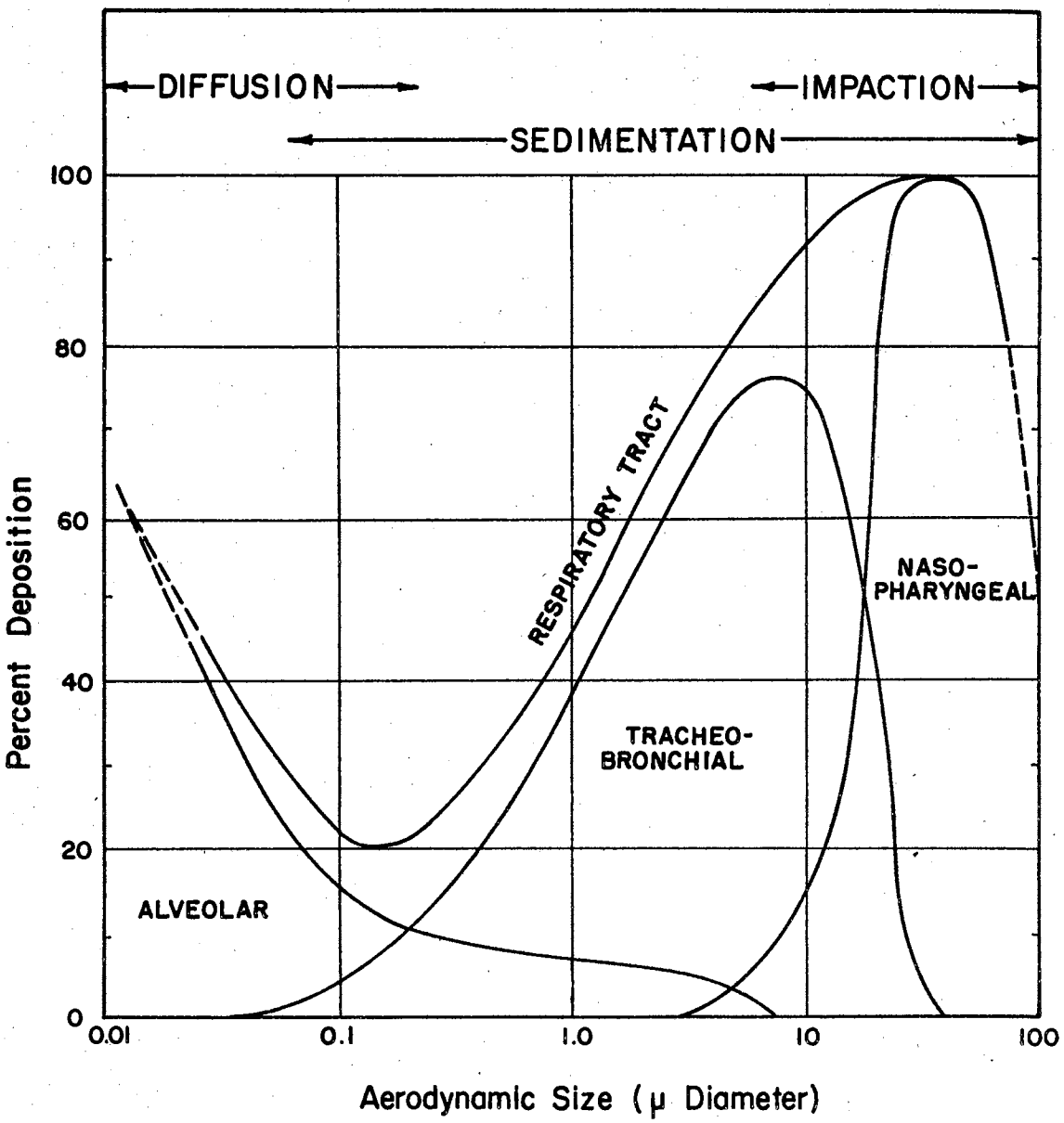

Figure 1 - Graphic summary of dust deposition in the respiratory tract.

bacilli. Their test showed a high rate of airborne bacteria from aeration units. The bacterial population ranged from eight per cubic foot on the upwind side, to 1170 per cubic foot on the downwind side. Their main concern was for the safety of plant operators and engineers. They concluded that the bacteria emitted from these units might be irritating to the respiratory tract. There was an attempt made to correlate the health of plant operators and the emission of airborne bacteria, but this failed due to the lack of personnel health records.

Napolitano and Rowe (12) conducted a study to compare the emissions of coliform organisms from activated sludge plants and high-rate trickling filter plants. This test was limited to coliform organisms collected for five-minute periods using an Andersen sieve sampler. The results indicated that the number of coliform organisms emitted from the aeration tank was ten times the amount from the high rate filter, and the high rate filter was twice the amount from the other units present. For a five-minute test at one liter per minute, the number of coliform organisms emitted from an activated sludge unit was 1139, compared with 149 from a trickling filter.

Higgins (13) conducted a model study of the survival of various bacteria under ideal conditions. This study controlled the environment and allowed for exact measurement of windspeed, humidity, temperature, and bacterial emissions. This research project consisted of a fan blow-

ing over an open source of bacteria and into a tunnel. The bacterial count was measured at various distances from emission. The results showed that coliform organisms were short-lived, and other bacteria were of more concern.

Webb (14) has conducted research on the ability of microorganisms to survive the effects of being air-dried. These tests showed that cells can remain viable after being air-dried, and that viability depends on the cells' combined response to relative humidity, temperature, and chemical additives. Hatch and Dimmick (15) studied the physiological responses of airborne bacteria to shifts in relative humidity. They concluded that bacteria have a high initial death rate upon becoming airborne, but this death rate decreases, and some cells were able to make readjustments until a suitable environment was reached.

Further research into the theories of airborne bacteria has shown the existence of other biological contaminants such as fungi, pollen, rickettsiae, and viruses (1). Respiratory diseases have become of great concern, because of the increased mortality rate attributed to upper respiratory diseases. Known bacteria cause about 8.2 per cent of the respiratory diseases, while 19.9 per cent are caused by virus and 71.0 per cent of unknown etiology (1). These respiratory diseases have alarmed the public, as shown by the organization of the U. S. National Health Survey and the increased concern of biological weapons in warfare (16). It has been determined that vegetative cells are the primary

etiological agents of communicable diseases. The pathogens of concern that resist drying are Staphylococcus, Streptococcus, and the Tubercle bacillus (16). Diseases attributed to airborne bacteria are scarlet fever, rheumatic fever, tuberculosis, Pneumococcus pneumonia, whooping cough, and meningitis (1). New research into the causes of disease has shown that many diseases known to have been transmitted by other means are also transmitted by air (1). It is now believed that Q fever found in ticks on cattle can be transmitted to man on dust, and gain entrance via the respiratory tract (1). As man increases his ability to collect and test airborne biological contaminants, more diseases of unknown routes of transmission will be attributed to airborne contaminants.

CHAPTER III

THEORETICAL CONSIDERATION

There are several possible methods of sampling for airborne bacteria. It is important to collect a representative sample of the bacteria under the conditions existing at the particular location and allow for further testing of the collected bacteria. The representative sample must provide the amount of bacteria per known volume of air. This sample must be collected at known time intervals for comparison with other conditions existing at the sampling location. The collected sample must remain viable for further testing and classification. Various means of collecting bacteria have developed through the past years. These range from open petri dishes to complicated commerical samplers. These samplers will collect the bacteria in the various quantities and conditions desired for the particular test.

Sedimentation

This is a simple means of collecting bacteria on particles that settle out of the air due to their specific gravity (5). They can be collected on watch glasses, filter papers, or in petri dishes containing a nutrient medium. This method of collection allows the samples to be incu-

bated and colonies counted corresponding to the exposure time. These samples will give a qualitative index of the bacteria suspended in the air, but no indication of the volume of air sampled. This method also eliminates the small particles that are influenced by air movement or Brownian movement (17). The collection surface can be placed at an angle to the direction of the wind, and the amount of bacteria collected will increase. Because the plate will be orientated in one direction, this method requires a high steady wind, and the particles still might miss the collection surface (3). This method is useful in the preliminary investigations of the bacteria present, but is very limited in its overall ability to provide a representative sample. Other means of collecting airborne bacteria are as simple to use, and they give a more representative sample per known volume of air sampled.

Sequential Sampler

The sequential sampler is used to collect bacteria on membrane filters. The air is drawn through each filter at a set rate of flow and length of time. This method can be used to collect bacteria on twelve filters for a total time of twenty-four hours. The membrane filter can be analyzed by direct counting with the aid of a microscope, incubated on a media and checked for colonies (18), or a protein stain applied to help determine protein particles in the air (19). This is a versatile method of collection that will collect a representative sample and allow testing for

the desired results.

Impingement in Liquids

This is a method of drawing air through a liquid medium that washes the bacteria from the air and suspends them in the liquid (16, 20). Figure 2 shows a schematic diagram used to collect airborne bacteria in a liquid medium. This sampler can be used in either of two ways. One method is to use a critical orifice with high velocities that prevents the bacteria from making a sharp turn, and suspends them in the liquid on impact. The other method is with low velocities that entrap the bacteria by percolation through the liquid. The volume of air sampled can be measured, and the time recorded. This method is probably not efficient in collecting bacteria smaller than five microns in size (16). These small bacteria can pass through the liquid in air bubbles. Since the nasal passage was established as the portal of entry and particles larger than five microns are filtered out by the nose and upper respiratory tract (10), the liquid impinger does not separate the bacteria into different size groups, and during the incubation the larger types of bacteria seem to predominate and prohibit the growth of the smaller bacteria (1). This method has been used (21), and after collection of the bacteria in the medium, a centrifuge was used for pre-separation of the media and bacteria. The sample was then filtered through a membrane filter for analyses. These tests were restricted to flows less than one liter per minute due to frothing of the liquid media.

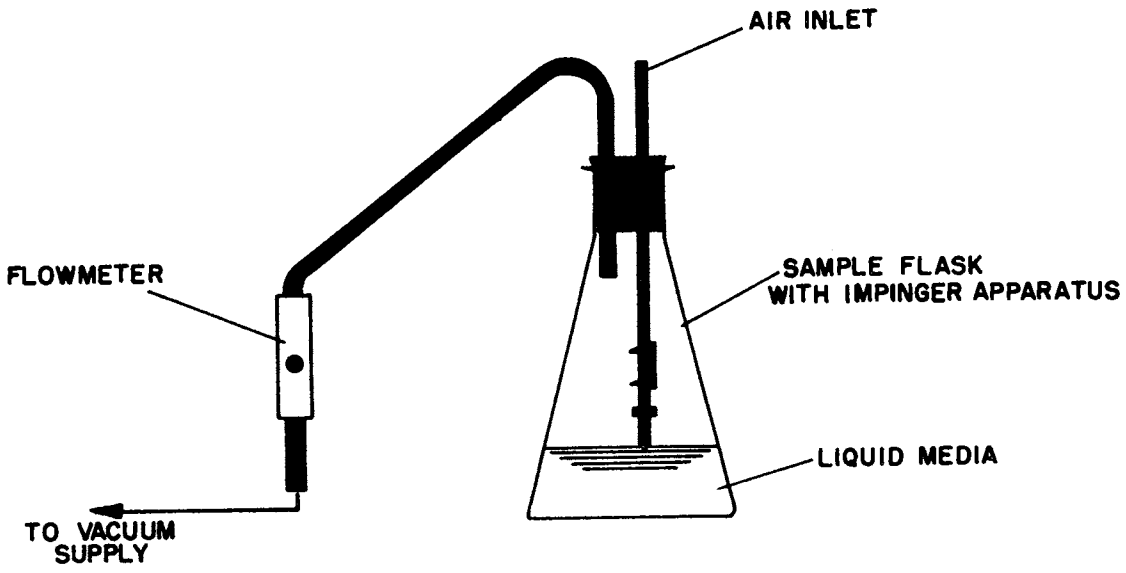

Figure 2 - Schematic diagram of liquid media sampler.

Impaction on Solid Media

This method has been used in several studies, because it allows for the collection of the desired size of bacteria while metering the volume of air sampled per unit of time. Several samplers are made that will provide a separate active growth of the individual bacteria colonies that allow counting per unit of time and various other tests (16). The impaction of the bacteria is accomplished by drawing an air sample through a critical orifice at a specified rate, and impacting it on the surface of a media. The Sentury sampler collects the bacteria on a single petri dish containing the solidified agar. This will collect most of the bacteria, but the small particles due to their small specific gravity can make the sharp turn and avoid impaction. The Andersen sieve sampler (22) will eliminate part of this problem by connecting six petri dishes in series. Figure 3 shows a schematic diagram of the six petri dishes and spacers. The air is drawn through spacer sieves containing 400 holes, and impacts the bacteria on the separate plates according to their aerodynamic size. This sampler collects various sizes of bacteria, but it has a limited surface area and should be used for short periods of time. The Andersen drum sampler (23) uses a critical orifice to impinge the bacteria on a drum that revolves around a center shaft, allowing for a spiral collection of 484 inches for various lengths of time. Figure 4 shows a schematic diagram of the drum sampler with its critical

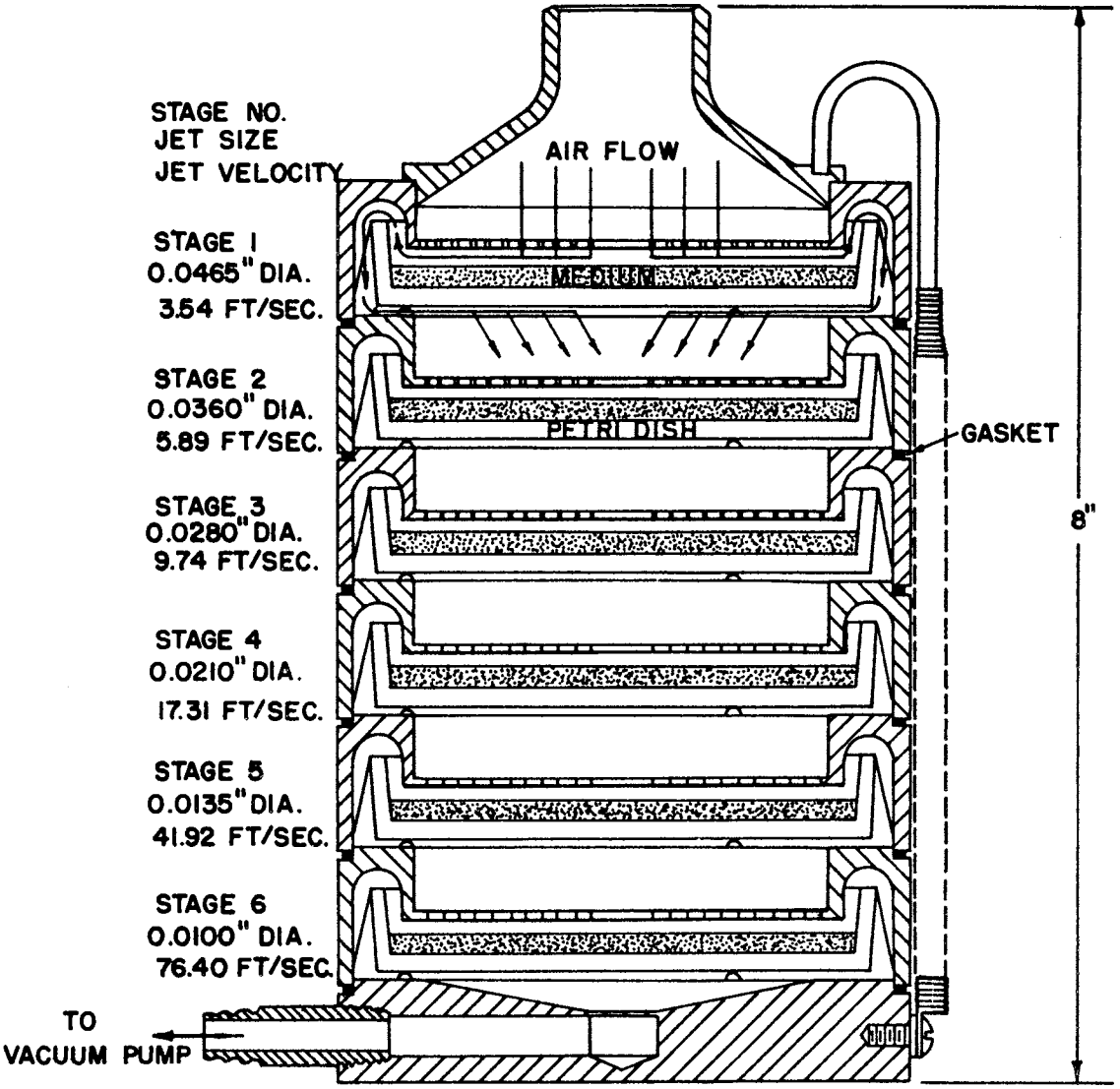

Figure 3 - Schematic diagram of sieve sampler.

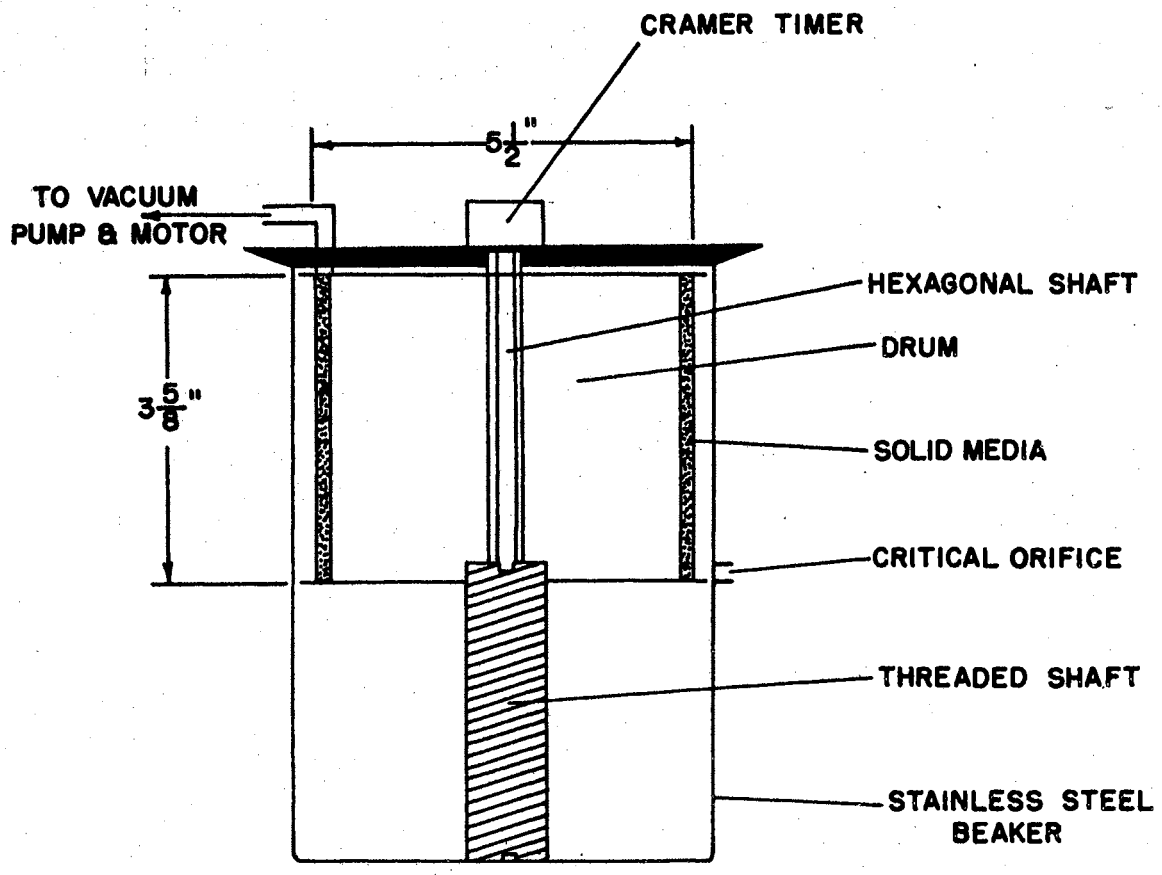

Figure 4 - Schematic diagram of drum sampler.

orifice. This will allow the collection of a representative sample of the desired bacteria that will remain viable for further testing.

CHAPTER IV

METHODS

The methods used in this research were oriented to collecting airborne bacteria under the actual conditions existing at the particular location. The sampling locations were selected to provide the desired units of aeration tanks and trickling filters. The methods used in sampling for the airborne bacteria were selected to simulate a person's breathing of bacteria, while the method of enumerating the bacteria was selected to represent the human body with respect to temperature and growth medium.

Locations

The tests for this project were conducted at the Stillwater sewage treatment plant and Oklahoma City Northside sewage treatment plant. These two plants were selected because of their volume of flow, type of treatments, and locations. Figure 5 shows a plan view of the Stillwater plant. The raw sewage flowed through a comminutor into a wet well, where it was pumped at a specific rate to the grit chamber. The sewage flowed over a weir and into the aeration tank. This was, in turn, pumped to the primary clarifiers. The sludge was pumped to the digesters, and

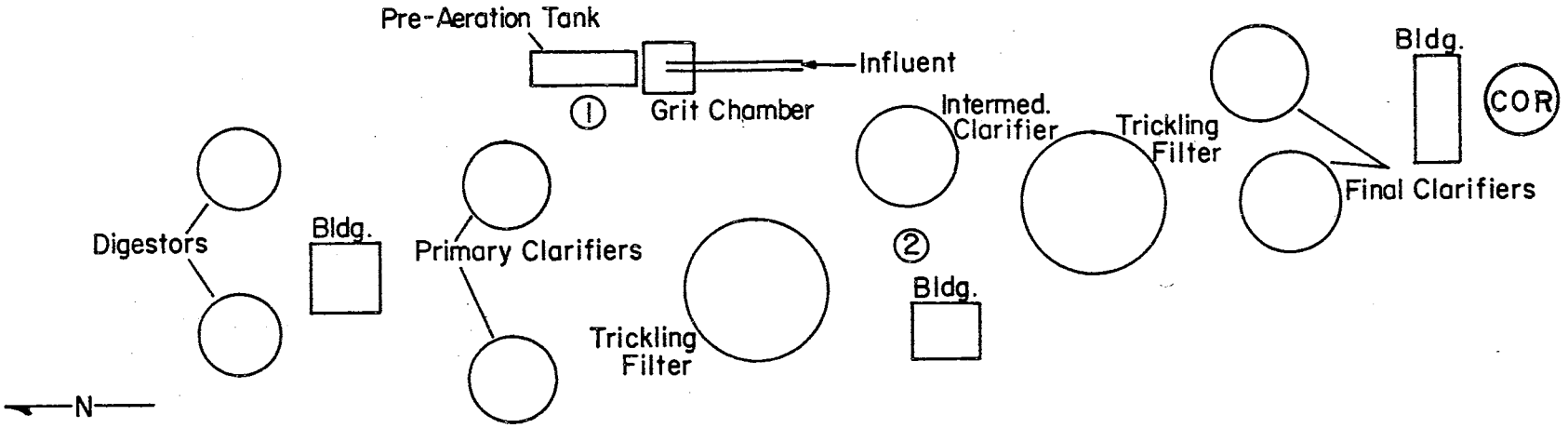

Figure 5 - Plan view of Stillwater Treatment plant and sampling locations.

the effluent flowed to the trickling filters. Two trickling filters were used in series with an intermediate clarifier. The effluent from the trickling filter was pumped to the final clarifiers and then flowed to a nearby stream. Figure 6 shows a plan view of the Northside plant. The raw sewage flowed into a grit chamber, where it flowed over a weir and through a comminutor. The sewage then flowed into the primary settling tanks, where the raw sludge was pumped to the primary digesters and the effluent flowed over a weir and into the aeration tanks. This, in turn, flowed to the final clarifiers, where the sludge was returned to the head of the aeration tanks for recirculation. These two plants provided a preaeration tank, two trickling filters, and an activated sludge unit for testing the emission of airborne bacteria. During the sampling program, control samples were collected at each plant to eliminate the background bacteria.

Media

The solid media used for testing airborne bacteria must allow the desired bacteria to grow upon incubation and remain viable, withstand pressures from impaction, not be influenced by atmospheric changes, and it should be easily reproducible (16). The air contains an enormous amount of bacteria, molds, yeast, and fungi that need not be tested, so a selective media was needed (1). The use of chemicals and stains prohibit the unwanted organisms, but extreme care must be used in testing the results of these media, for they may prohibit or retard the growth of wanted bacteria.

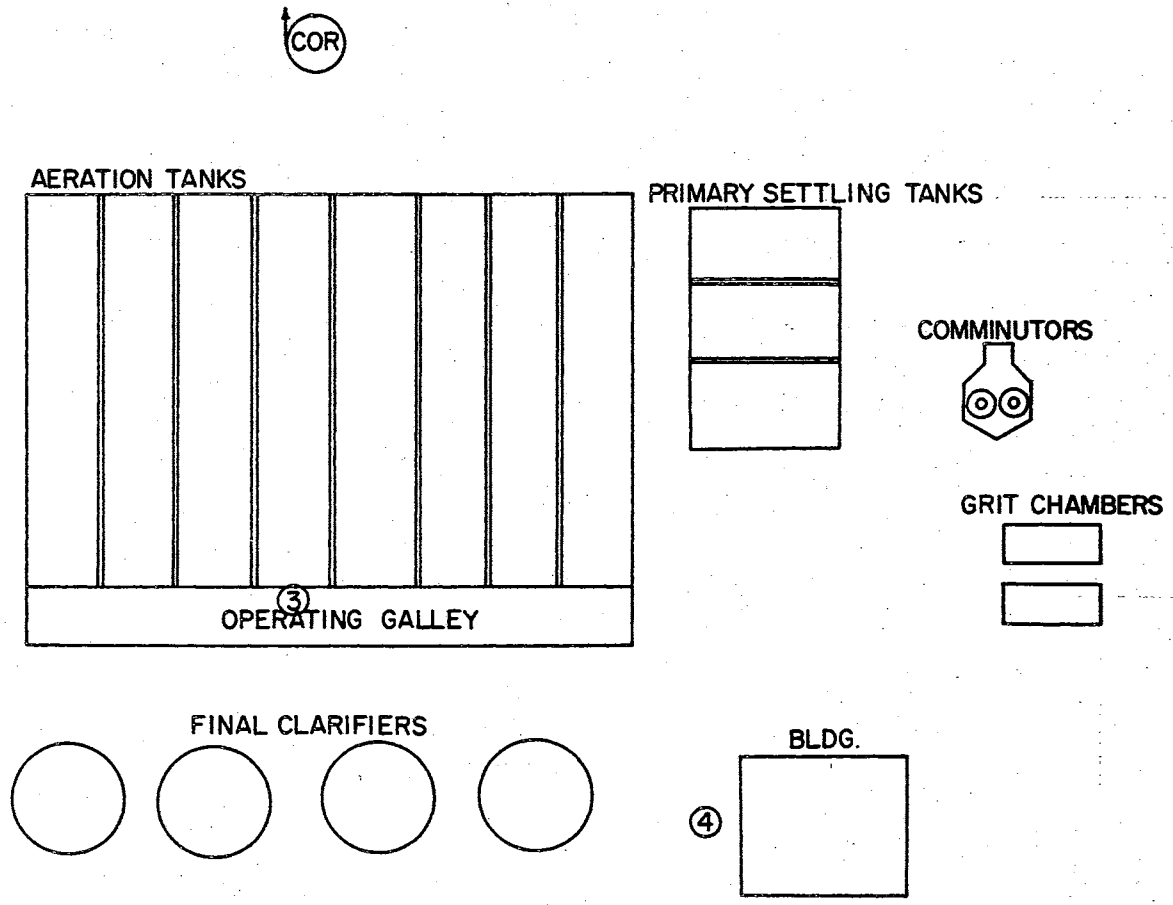

Figure 6 – Plan view of Oklahoma City Northside treatment plant and sampling locations.

These formulas can be found in microbiology texts and laboratory manuals (1, 16, 18, 20). Most of the formulas listed can be purchased in a commercial dehydrated form, which eliminates the weighing of each ingredient and gives a more uniform media with reproducibility. These media were prepared by weighing the amount of media required for the specified volume of distilled water. This mixture was agitated under a low flame until it became a solution. The media solution was autoclaved for sterilization and poured into the molds, petri dishes, or stored in a water bath at a temperature above the solidification point until needed.

The media tested for this project were nutrient agar, starch agar, lactose broth, and eosin methylene blue agar. The formulas for the above media are listed in Table I, on page 24. Several preliminary tests were conducted to determine the sterility of the poured plates and the reaction of the solid media to the impaction of the air.

Samplers

The Sentury sampler (Figure 7) was employed to make a preliminary test of the media and procedure. Various types of agar were prepared and tested to see if viable bacteria could be collected. The results of this test are listed in Table II, on page 26 of the text.

The sequential sampler (Figure 8) was used to collect the various particles in the air on membrane filters. Two-hour samples were collected on twelve separate filters. This gave a total testing time of twenty-four hours, and a

TABLE I

THE FORMULAS FOR MEDIA USED IN GRAMS PER LITER

Nutrient agar (NA), 31 grams
- Peptone, 5
- Beef extract, 3
- Sodium chloride, 8
- Agar, 15

Starch agar (SA), 25 grams
- Peptone, 5
- Beef extract, 3
- Soluble starch, 2
- Agar, 15

Tryptose agar (TA), 41 grams
- Tryptose, 20
- Glucose, 1
- Sodium chloride, 5
- Agar, 15
- Thiamine hydrochloride, 0.005

Blood agar (TAB), 50 ml/1000 ml
- 5% blood added to the media base used

Lactose broth (LB), 13 grams
- Peptone, 5
- Beef extract, 3
- Lactose, 5

Eosin methylene blue agar (EMB), 37.5 grams
- Peptone, 10
- Lactose, 10
- Dipotassium phosphate, 2
- Eosin y, 0.4
- Methylene blue, 0.065
- Agar, 15

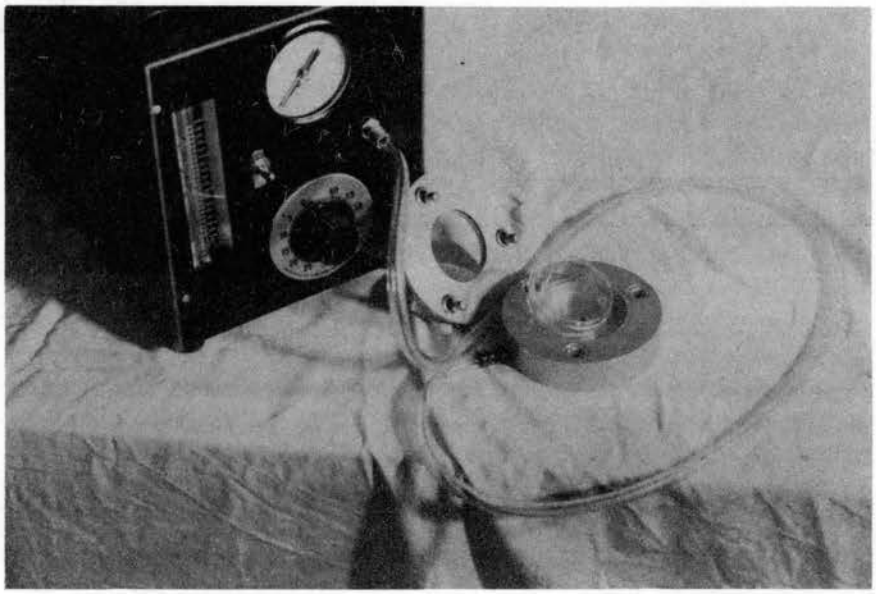

Figure 7 - Sentury sampler.

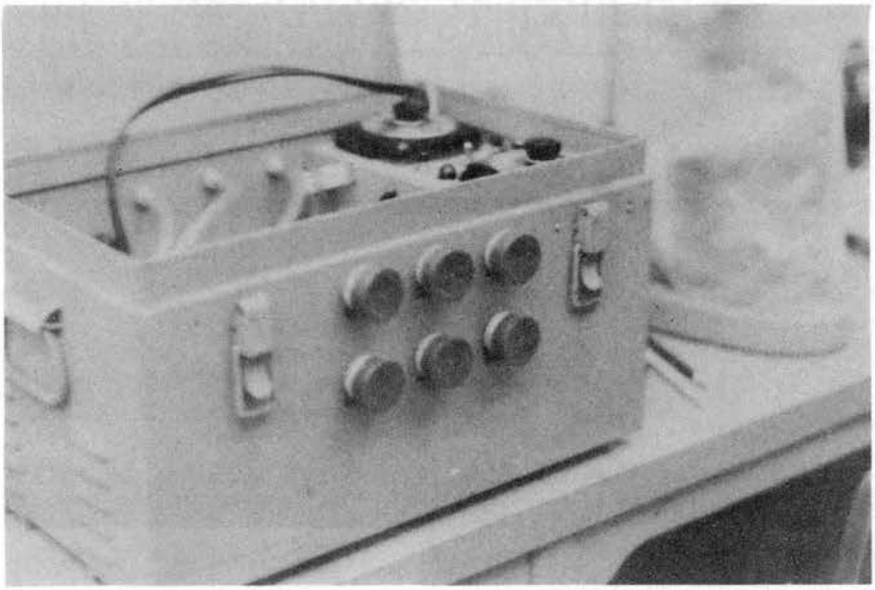

Figure 8 - Sequential sampler.

TABLE II

PRELIMINARY TEST RESULTS

Sample	Site	Time	Colonies	Sample	Site	Time	Colonies
NA	Lab.	1230	35	NA	Lab.	1230	45
SA	Lab.	1230	5	SA	Lab.	1230	10
NA	Lab.	1230	25	NA	Lab.	1230	20
NA	Lab.	1230	11	NA	Lab.	1230	25
TA	Plant	1000	240	TA	Plant	1010	200
TAB	Plant	1020	6 Hemo.	TAB	Plant	1030	4 Hemo.
TA	Plant	1100	220	TA	Plant	1110	240
TAB	Plant	1120	Hemo.	TA	Lab.	1100	24
TA	Lab.	1100	7	NA	Lab.	1200	7
NA	Lab.	1300	6	NA	Lab.	1400	12
NA	Plant	1000	70	NA	Plant	1030	TNC*
TA	Plant	1400	53	TA	Plant	1530	15
SA	Plant	1530	TNC*				

*TNC = too numerous to count.

The samples were collected using the Sentury sampler for ten minutes at twenty liters per minute (LPM), making a volume of 200 liters. The temperature in the laboratory was 24°C., and the temperature at the plant varied between 23°C. to 25°C. The samples were NA (nutrient agar), SA (starch agar), TA (tryptose agar), and TAB (tryptose blood agar).

representative sample of airborne bacteria. This sampler was located by the aeration tank and activated sludge unit to determine if bacteria were being emitted from these units.

The sieve sampler (Figures 9, 10) was used to collect the bacteria for short periods of time. These samples were collected downwind of the units, while a corresponding control sample was being collected upwind of the units. The samples were conducted for ten minute duration, using petri dishes containing tryptose agar as the sampling medium. The petri dishes were numbered and returned to the laboratory for analysis.

The drum sampler (Figures 11, 12) was used because it would sample continuously. The 27-hour timer allowed for long periods of testing, and gave a representative sample. The drums were placed next to the units, and one sampler was placed upwind of the units for a control. The drums spiraled down and collected the sample on the 484 inches of available space. The drums contained tryptose agar as a collection medium. This medium contained two per cent higher concentration of agar to withstand the impaction action. Strips of wet sponge were kept in the drum samplers to help the collected bacteria remain viable.

Aerometric Measurements

The collection of a representative sample was important but in order to understand the variation of the bacterial population, other data were recorded. The starting and

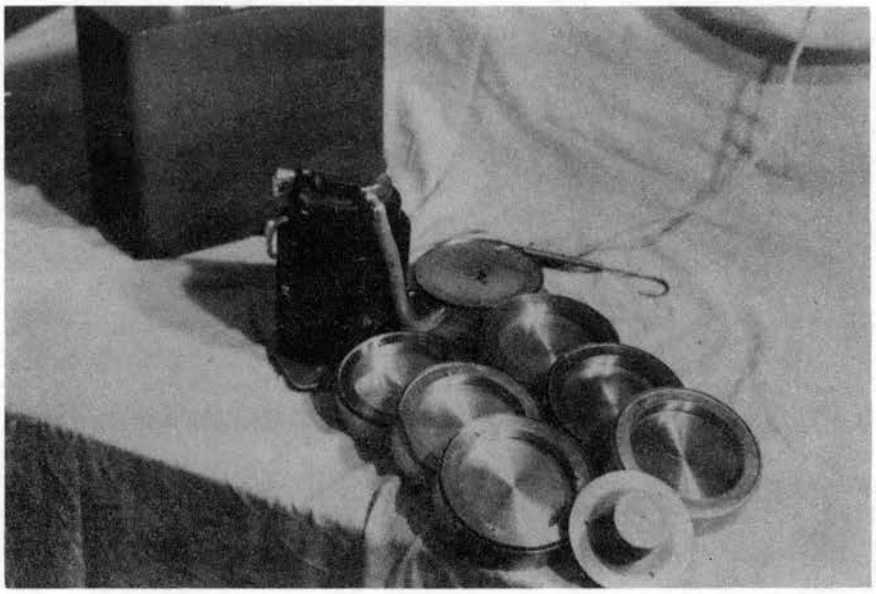

Figure 9 - Andersen sieve sampler (unassembled).

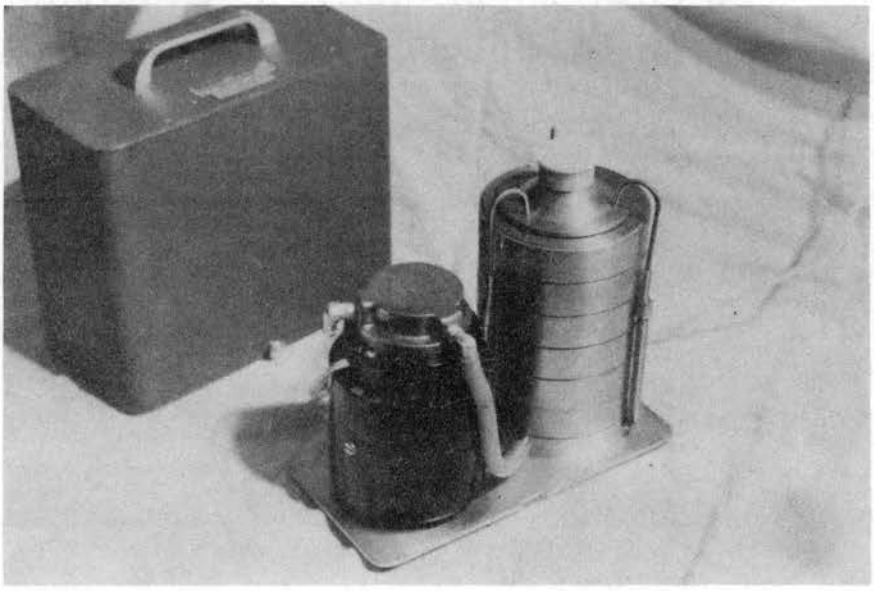

Figure 10 - Andersen Sieve Sampler

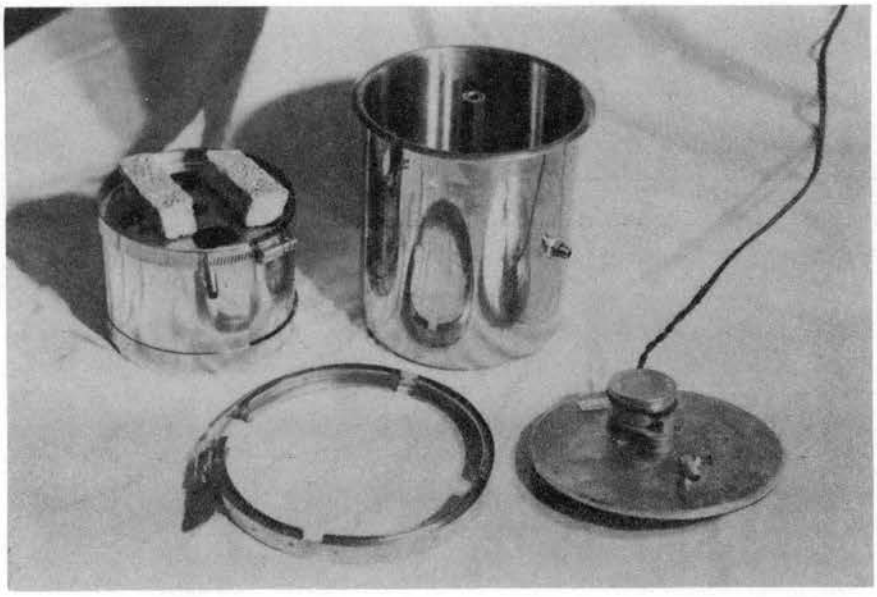

Figure 11 - Andersen drum sampler (unassembled)

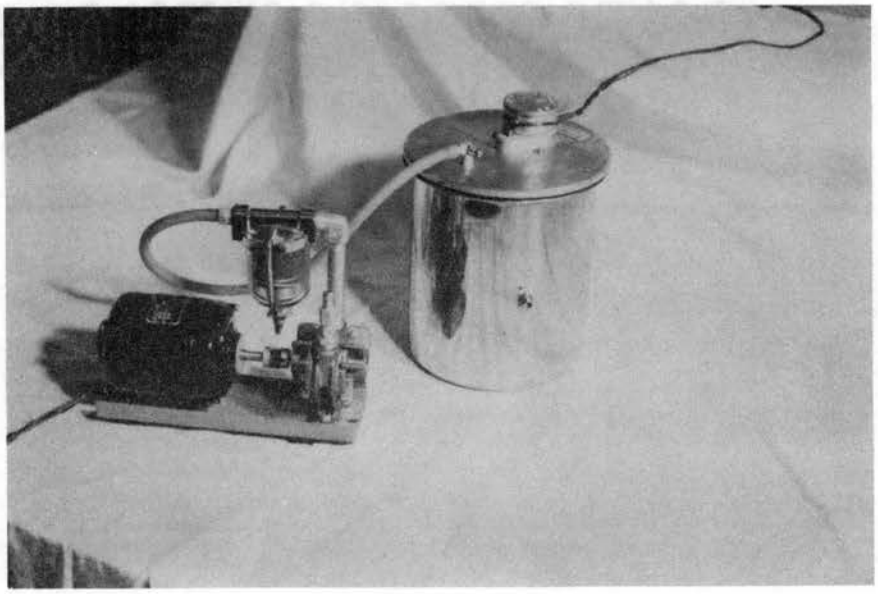

Figure 12 - Andersen drum sampler.

ending time of each sample was recorded to allow comparison with other data collected. The sewage flow and ambient temperature were taken from the daily log kept by the plant operators. The wind speed and relative humidity were taken from airports near each plant location. Stillwater Airport takes hourly readings of wind direction and speed, dry-bulb temperature, and dew point. The relative humidity was calculated by plotting the dry-bulb temperature against the dew point on a psychrometric calculator. Will Rogers World Airport takes hourly readings of wind speed and direction, temperature, and relative humidity. These relative humidity readings were taken from a hair-hygrograph. This data was collected according to the starting and ending time of each sample.

Sample Analysis

After collection, the samples were returned to the laboratory for analysis. Membrane filters collected with the sequential sampler were stained by using a protein stain. The filters were stained by placing an absorbent paper pad in each of four petri dishes. Sufficient reagents were added to the petri dishes to saturate the pad without immersing it. These reagents were A (nitric acid), B (ninhydrin), C (pink RL), and D (ethyl alcohol). The filters were placed deposition side up on each absorbent pad for two minutes, and blotted on the bottom side after each treatment. The filters were then oven-dried for ten minutes at 37°C. Transparent slides were made of one-

fourth of the stained filter by clearing the filter with immersion oil. The protein content was then determined microscopically at 970 x magnification. The stain colored the protein in the bacteria pink. The bacterial content was observed by comparing the morphology of the bacteria and other protein particles present.

The bacteria impacted on the tryptose agar petri dishes and drums were incubated as soon as possible after collection. It was important to keep the collected organisms viable. The selection of the proper physical environment was as important as the selection of the proper nutrient. The optimum temperature varied considerably for each organism, but since the host for the bacteria was the human body, 37°C. was used as the incubation temperature. Preliminary samples were incubated for forty-eight hours, and the colonies were counted at twelve-hour intervals. There were no additional colonies appearing after twenty-four hours of incubation. During incubation periods longer than twenty-four hours, the colonies spread together and lost their identity. Therefore, incubation time was limited to twenty-four hours at 37°C. The bacteria collected by the sieve sampler on the six petri dishes were counted and recorded by sieve size (Figure 13). The number of colonies counted on each stage of the control sampler was subtracted from the number of colonies counted on the corresponding sample collected downwind of the unit. The bacteria collected on the drum sampler were counted after

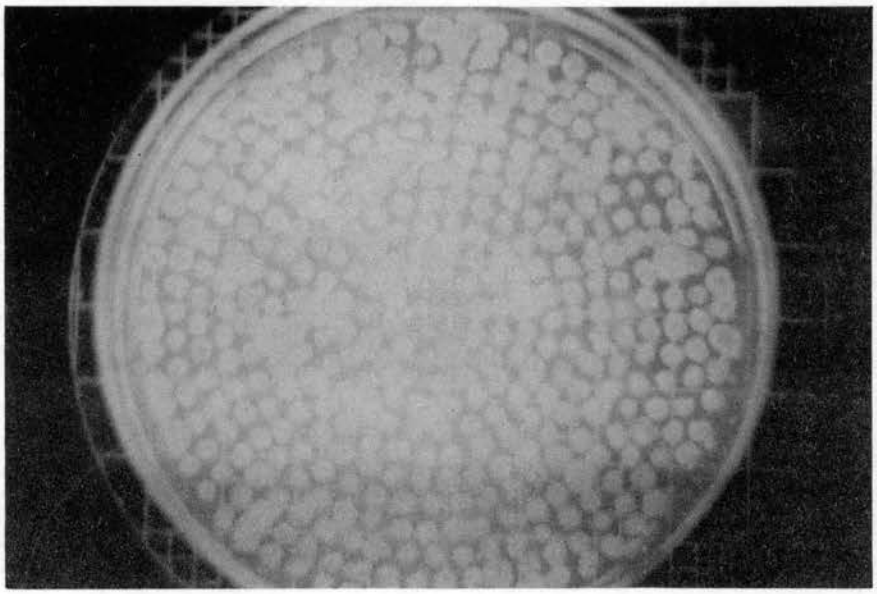

Figure 13 - Colonies collected by sieve sampler.

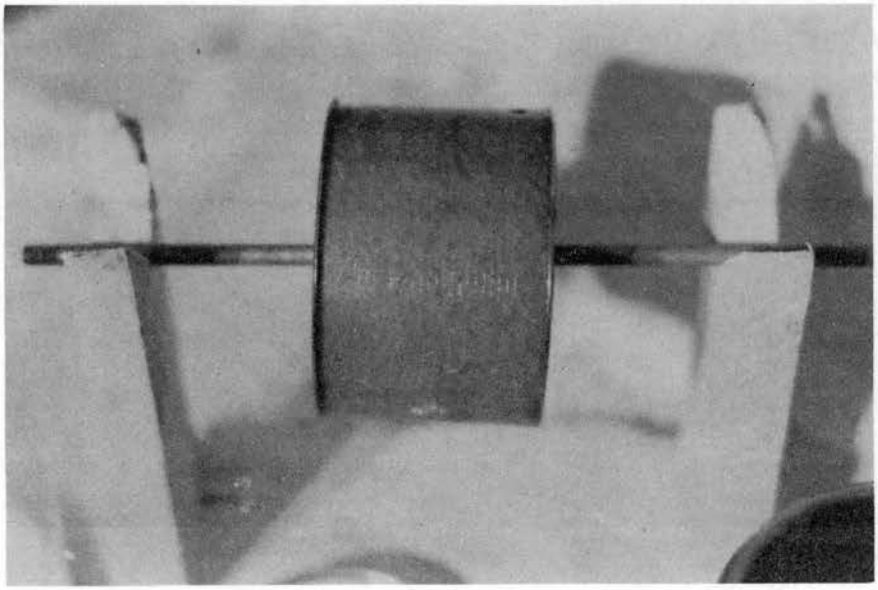

Figure 14 - Colonies collected by drum sampler.

incubatiom (Figure 14). The hourly number of colonies was obtained by the number of colonies counted in 1/20 of a revolution less the control count, and multiplied by 20.

The collected bacteria were not tested for identification, but a series of tests (Figure 15) were performed on twenty colonies collected by the drum sampler to determine their probable viability, reaction with blood, and some means of classification. The entrapped bacteria had to reproduce on agar slants in order to be able to assume they were viable and able to reproduce in the human body. If they remained viable, they were streaked on tryptose blood agar plates and after incubation were checked for hemolysis. The classification of the bacteria was concluded by their reaction to eosin methyelene blue agar and gram stain. Slides were prepared, and a microscope (Figure 16) was used to determine the staining characteristics and the morphology of the bacteria.

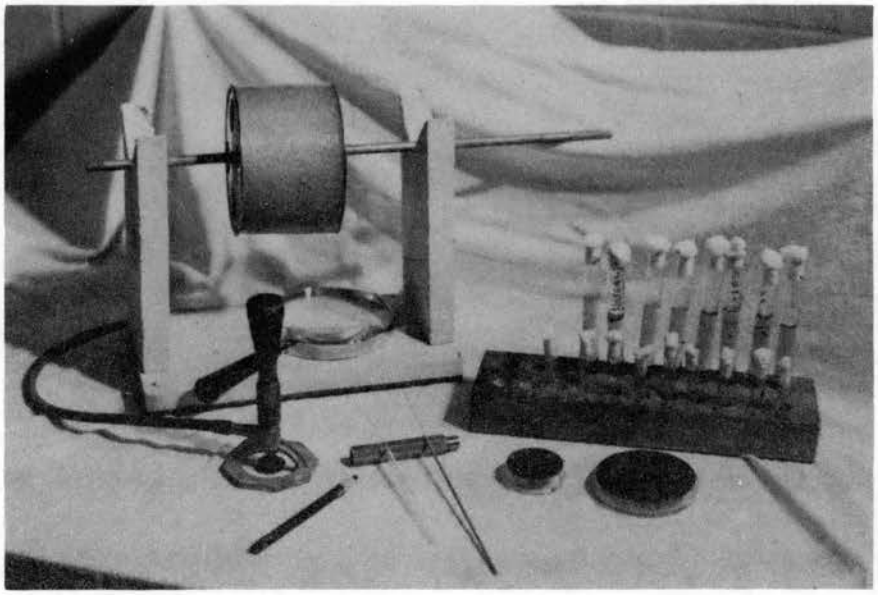

Figure 15 - Test for bacterial analysis.

Figure 16 - Microscope for determining morphology.

CHAPTER V

RESULTS AND CONCLUSIONS

This research was undertaken to determine if airborne bacteria were being emitted from sanitation treatment units. Tests were conducted next to a pre-aeration tank, trickling filter, activated sludge unit, and two control stations. The results of these tests were analyzed and a comparison was made between the amount of bacteria emitted from each unit, bacterial count above control to parameters influencing airborne bacterial survival, and the classification of the bacteria emitted. The pre-aeration tank, trickling filter, and control one located at Stillwater sewage treatment plant (Figure 5) and the activated sludge unit and control two located at Oklahoma City Northside treatment plant (Figure 6) were used for this research. Figures 17 and 18 show the drum, sieve, and sequential samplers in operation at the pre-aeration tank. Figure 19 shows the drum sampler with the trickling filter in the background. Figure 20 shows the drum and sieve samplers at control one. Figure 21 shows the activated sludge unit with the sieve and drum samplers at the opposite end. Figure 22 shows the sieve and drum samplers at control two. This research was

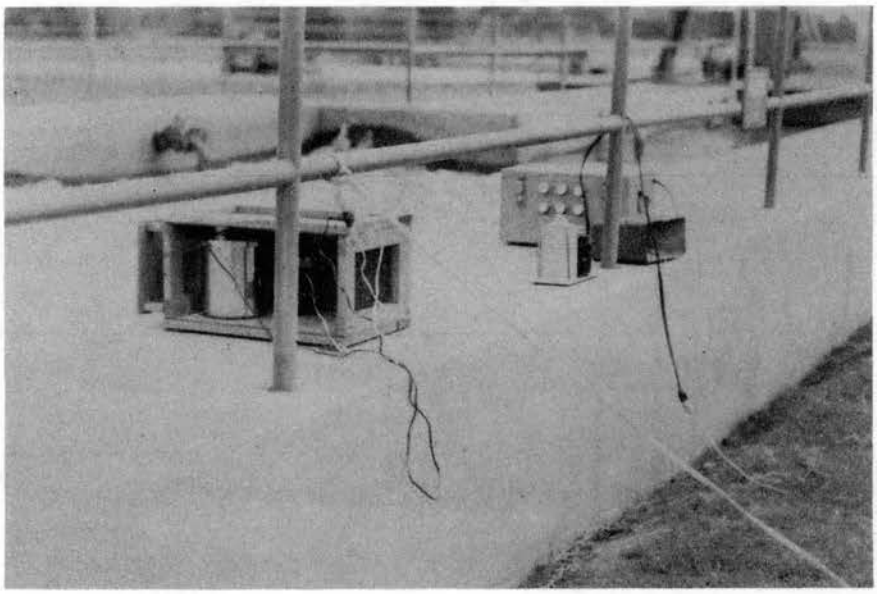

Figure 17 - Drum, sieve, and sequential samplers in operation.

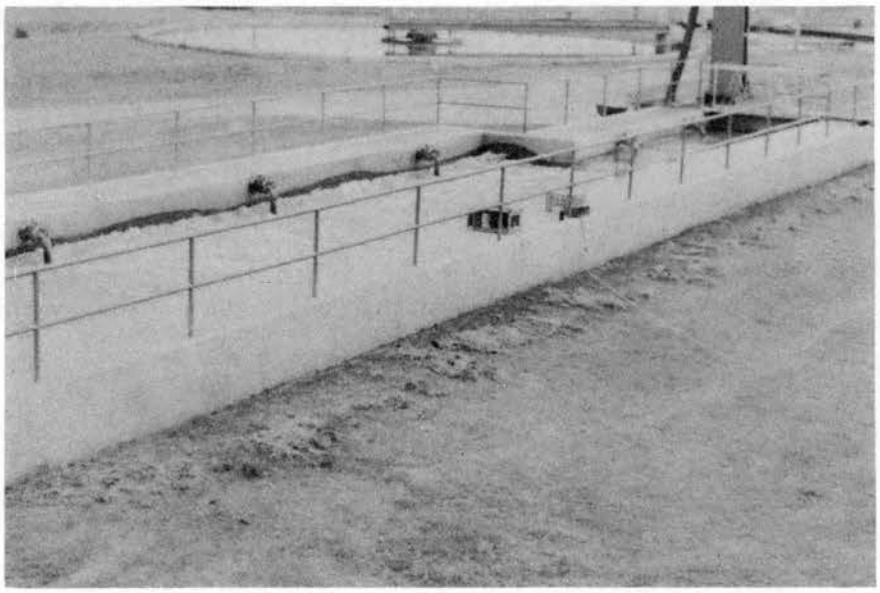

Figure 18 - Testing at pre-aeration tank.

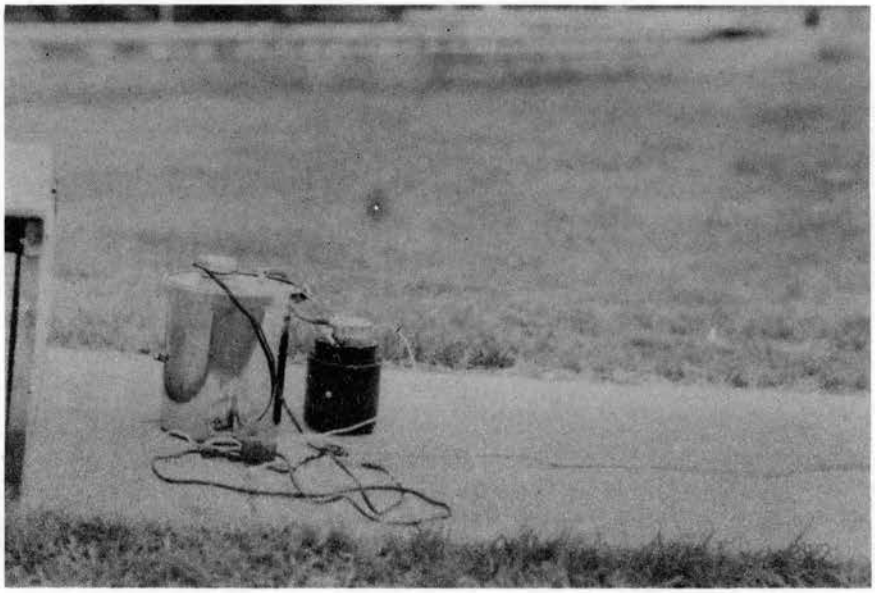

Figure 19 - Drum sampler near trickling filter.

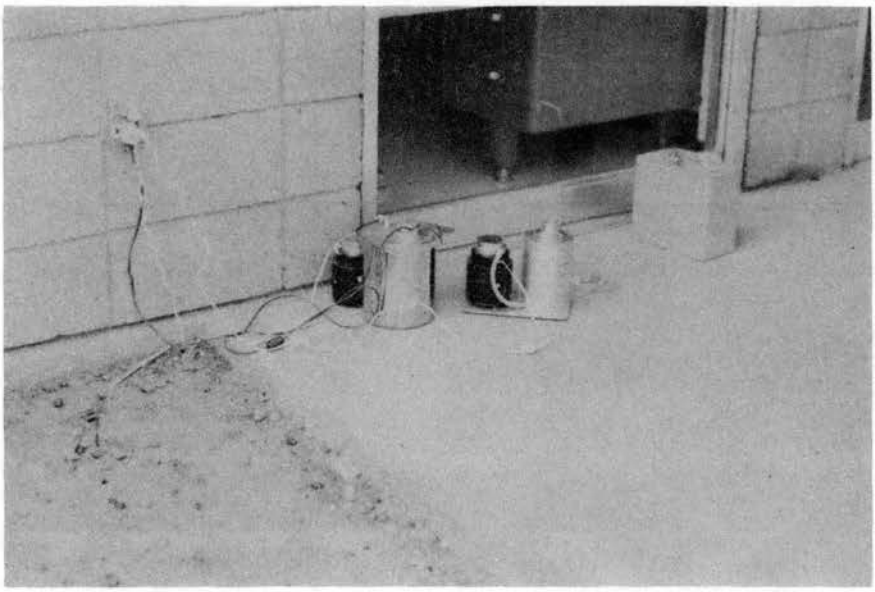

Figure 20 - Drum and sieve sampler at control one.

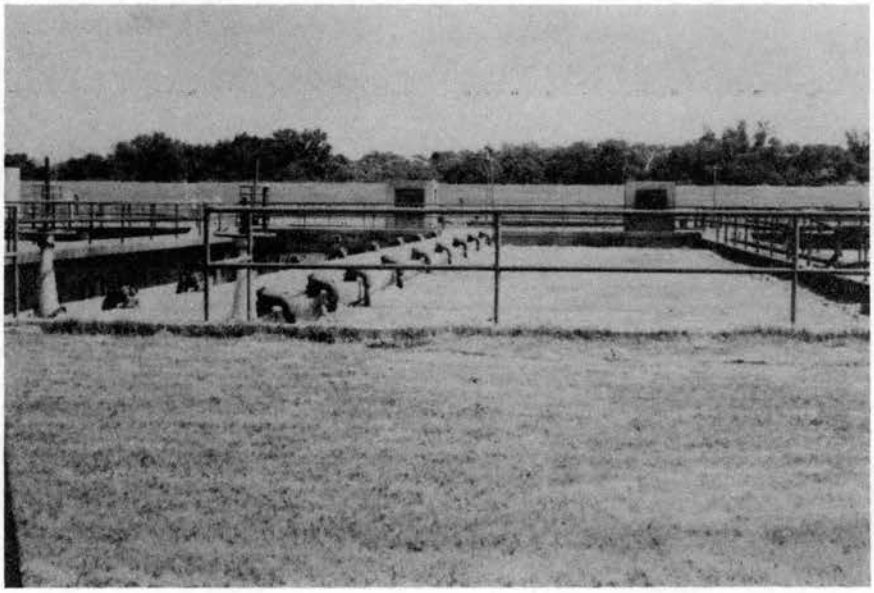

Figure 21 - Activated sludge unit.

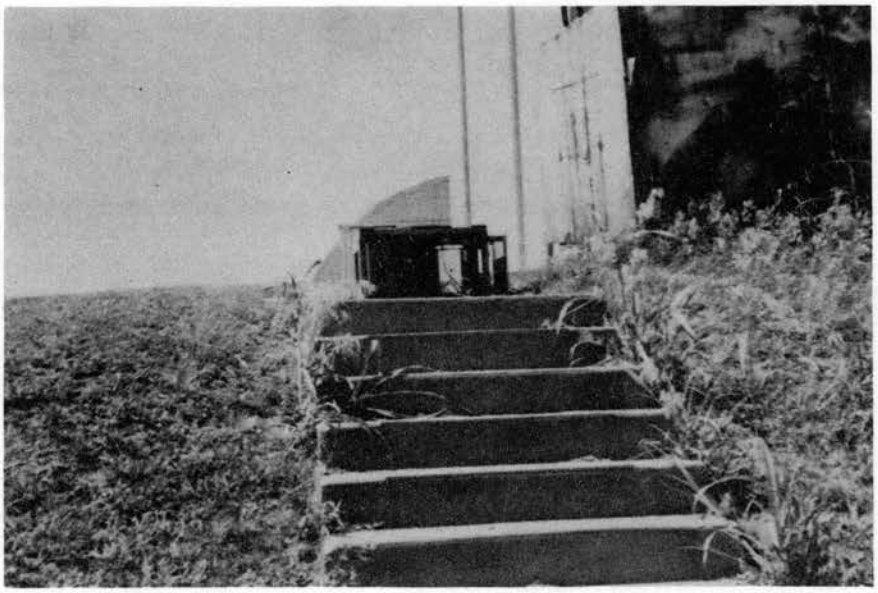

Figure 22 - Drum and sieve sampler at control two.

concluded with a preliminary investigation using a tracer bacteria placed in the sewage influent. This test was conducted to determine if a known bacteria could be collected in the air emitted from the pre-aeration tank.

Bacterial Emission

The membrane filter samples collected with the aid of the sequential sampler were analyzed, and the results show that bacteria were being emitted from the pre-aeration tank and the activated sludge unit. Because a sequential sampler was not used for control, no comparison was made between these two units.

The sieve samplers were operated at one cubic foot per minute, while the drum samplers were operated at one liter per minute. The samples were analyzed at one cubic foot for comparison. Table III shows the quantitative comparison between the sieve and drum sampler and the different units tested.

TABLE III

QUANTITATIVE COMPARISON OF BACTERIAL EMISSION

Location	Sampler	No. Colonies per cu.ft. of air
control one	sieve	80
	drum	28
control two	sieve	86
	drum	40
pre-aeration tank	sieve	173
	drum	351
activated sludge	sieve	146
	drum	93
trickling filter	sieve	no results
	drum	77

The number of colonies collected per cubic foot of air was higher using the sieve sampler than the drum sampler, except for the tests conducted at the pre-aeration tank. At this location, the drum sampler collected twice the number of colones as the sieve sampler. These results can be explained by comparing the methods of testing. The sieve sampler was used for ten-minute time intervals and could have collected the bacteria when other parameters were causing an unknown high or low rate of emission. The drum sampler collects the bacteria continuously for long periods of time through changing parameters, and gives a more representative count of bacterial emission. Comparing the bacterial emission rate for all units tested, the pre-aeration tank was the highest, followed by the activated sludge unit and trickling filter. These comparisons indicate that bacteria were being emitted from the pre-aeration tank, trickling filter, and activated sludge unit.

Parametric Effects

The amount of bacteria above controls that was emitted from each unit was compared with the independent variables of sewage flow, ambient temperature, wind speed, and relative humidity. The results were plotted, and the linear regression equation for each parameter was calculated. The model was defined as:

$$\hat{Y} = \hat{B}_o + \hat{B}_1 X$$

where

$\hat{Y}$ = the expected number of bacteria

$\hat{B}_o$ = Y intercept

$\hat{B}_1$ = slope of line

X = independent variable

The results of these parametric effects and the equation of linear regression are shown in Figures 23 through 34. These figures show the parametric effects as the independent variable, and the number of colonies above control per hour as the dependent variable. The data used to calculate the linear regression were grouped and plotted, with the number of sampling points in each group shown at the bottom of the figure. The wide dispersion shown in these figures indicates that there were other parameters influencing the rate of bacterial emission. Figures 24 through 26 show a higher rate of colonies emitted from the pre-aeration tank for the four parameters considered, as compared with the trickling filter and activated sludge unit (Figures 27 through 34). Figures 23, 27, and 31 show little influence on the number of colonies emitted attributable to sewage flow through the plant. The slopes for these linear regression equations were all positive; that is, the number of bacteria tended to increase as flow increased. Figures 24, 28, and 32 indicate a change in the number of colonies emitted by temperature. The slopes for the linear regression lines in Figure 24, the pre-aeration tank, and Figure 28, the trickling filter, were negative,

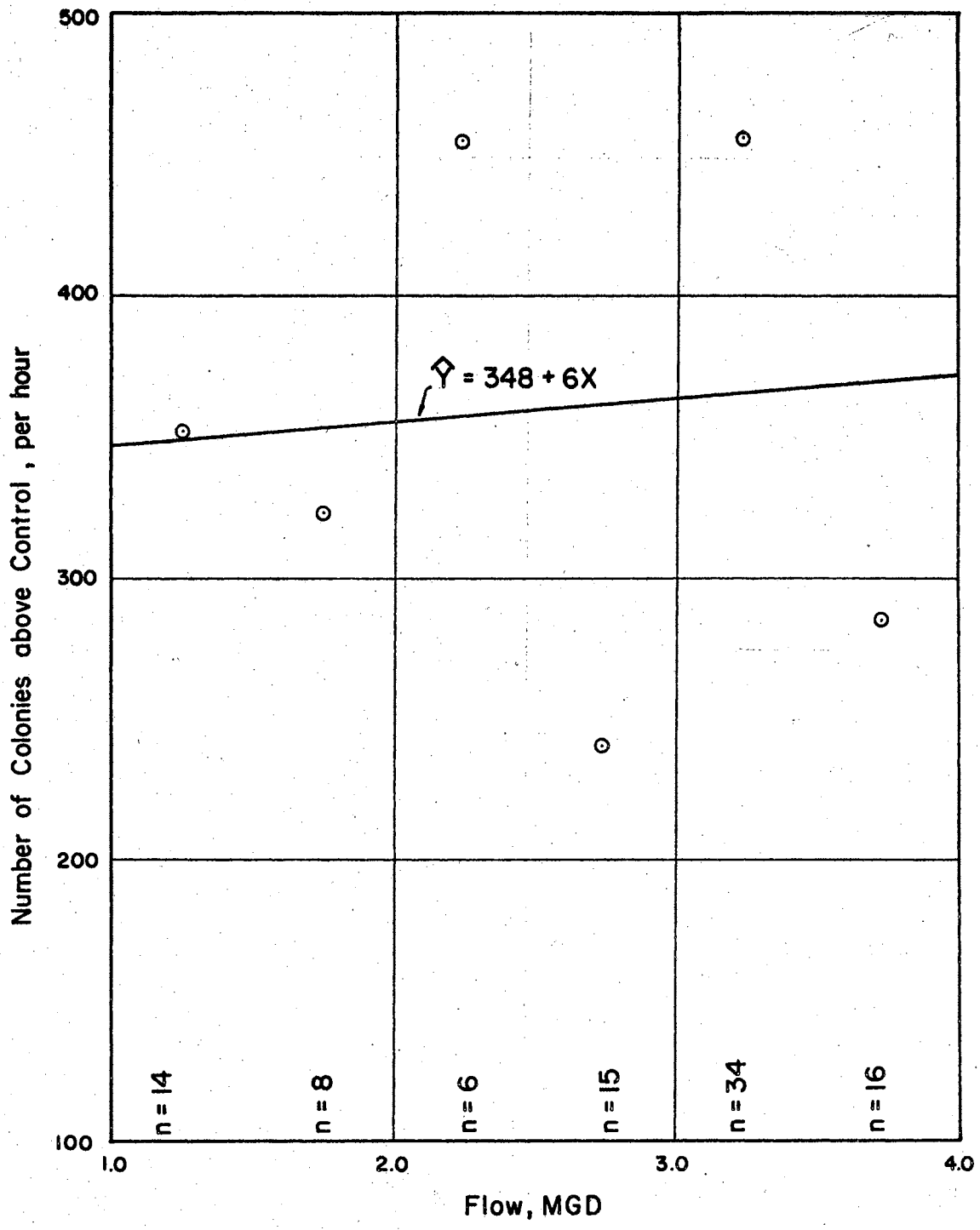

Figure 23 - Relationship between bacterial colonies and flow - pre-aeration tank.

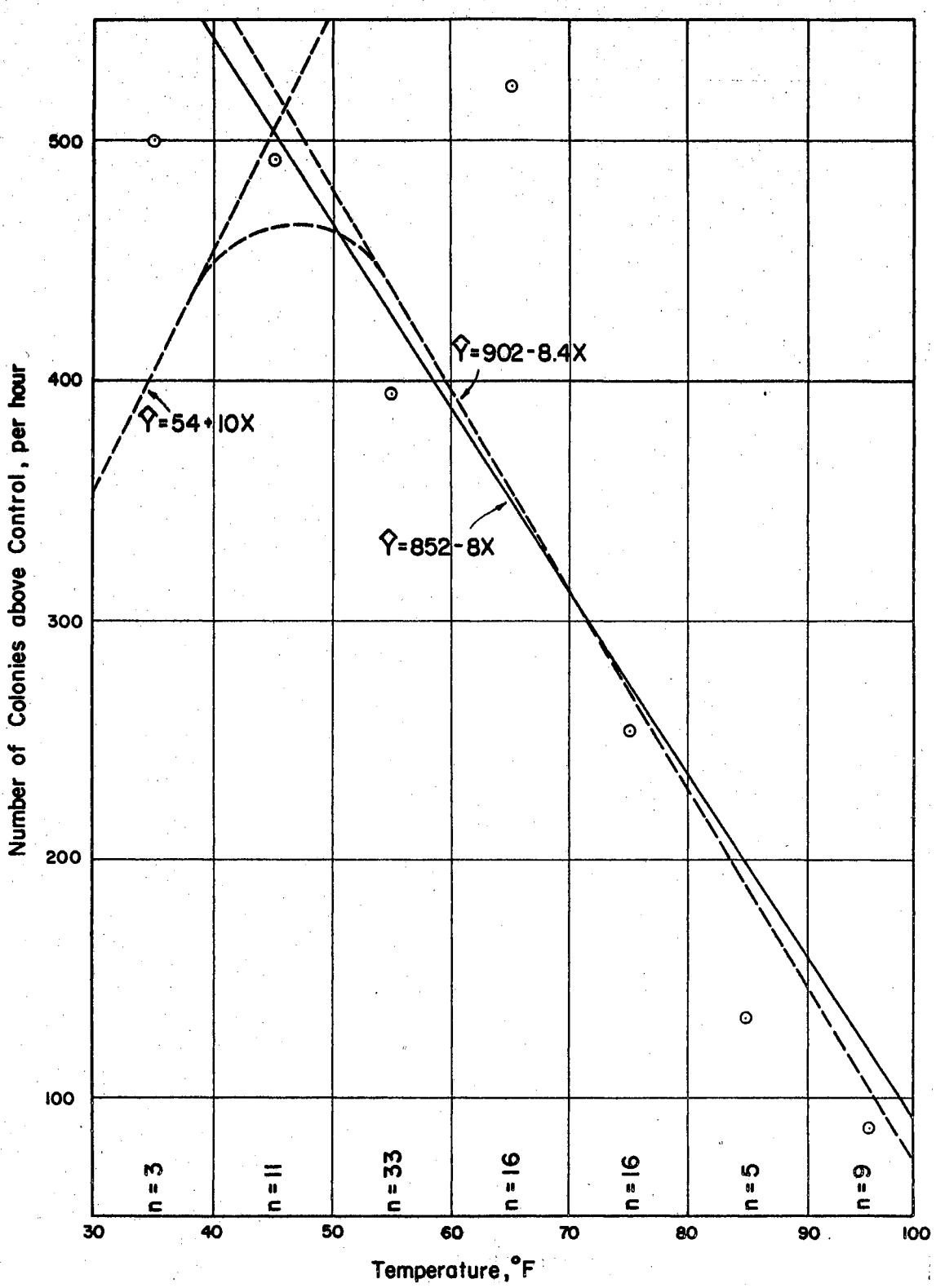

Figure 24 - Relationship between bacterial colonies and temperature - pre-aeration tank.

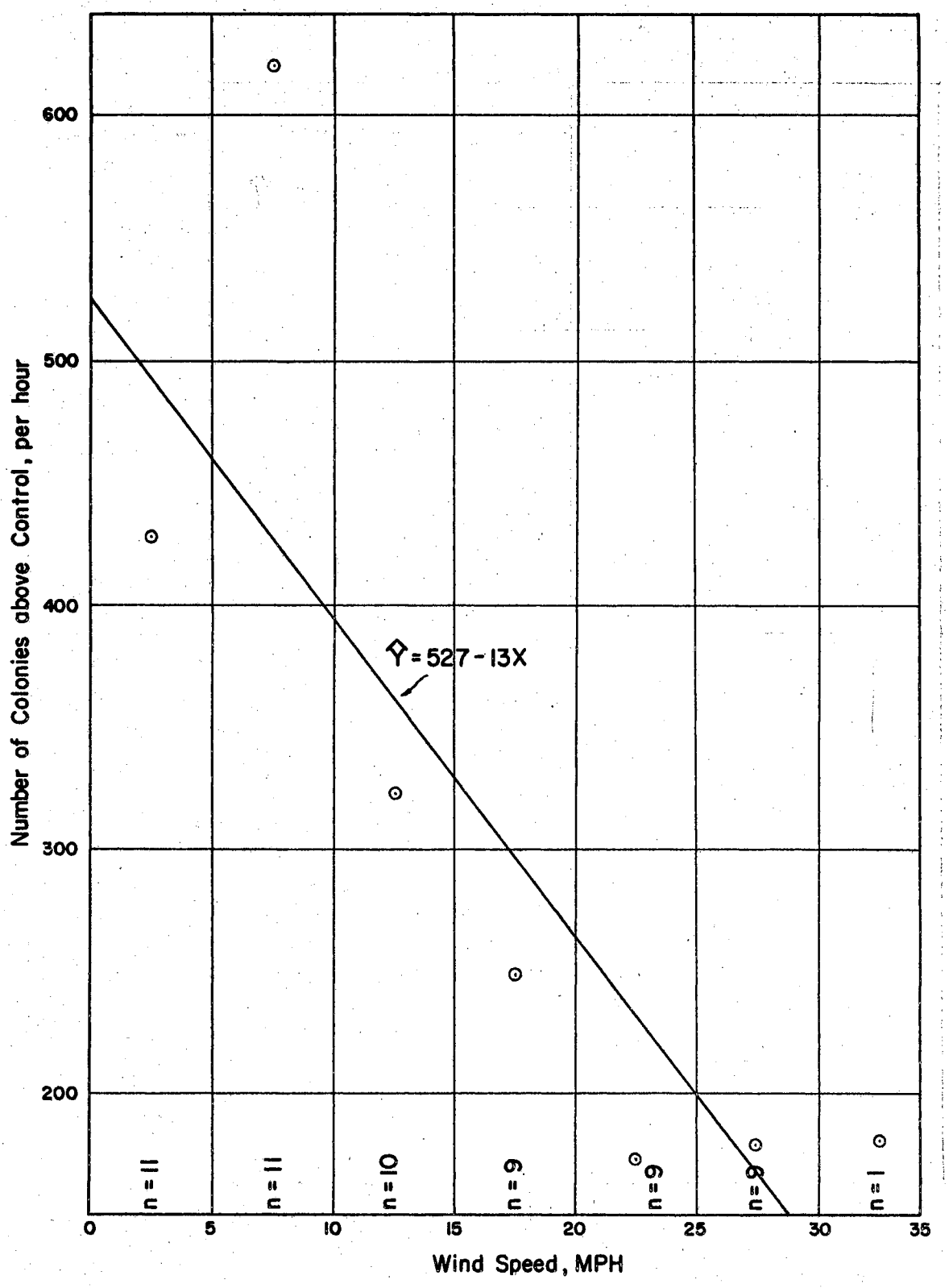

Figure 25 - Relationship between bacterial colonies and wind speed - pre-aeration tank.

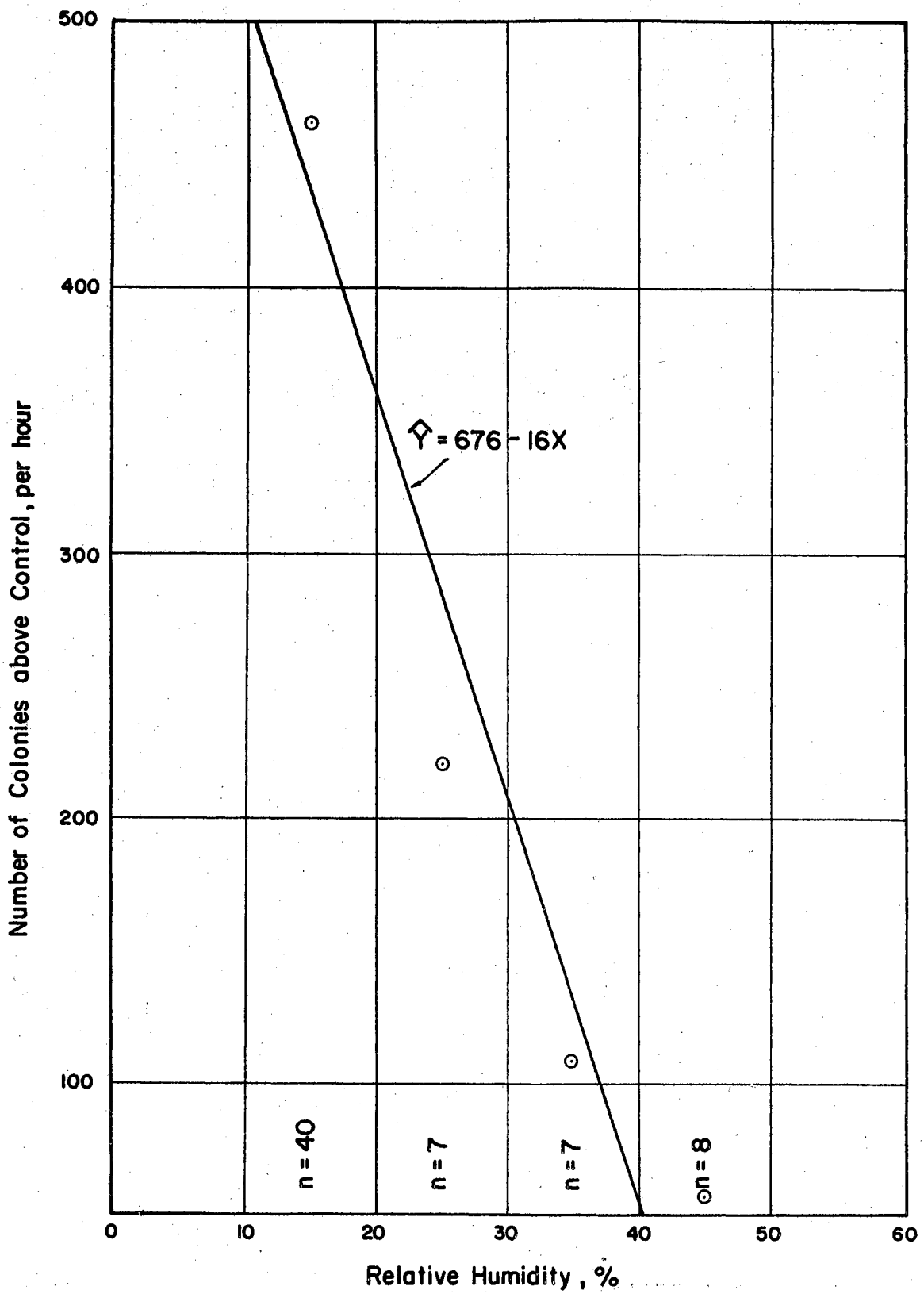

Figure 26 - Relationship between bacterial colonies and relative humidity - pre-aeration tank.

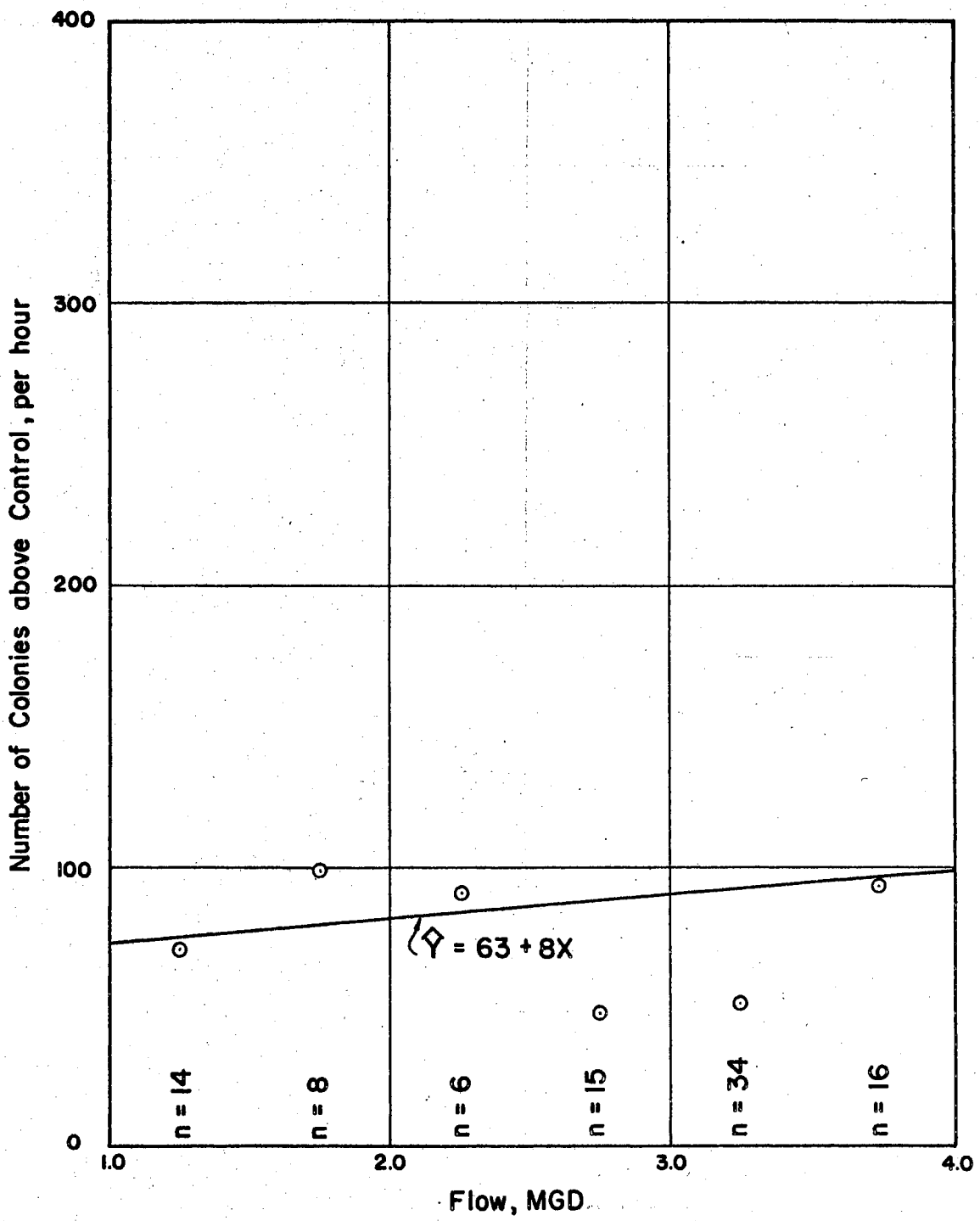

Figure 27 - Relationship between bacterial colonies and flow - trickling filter.

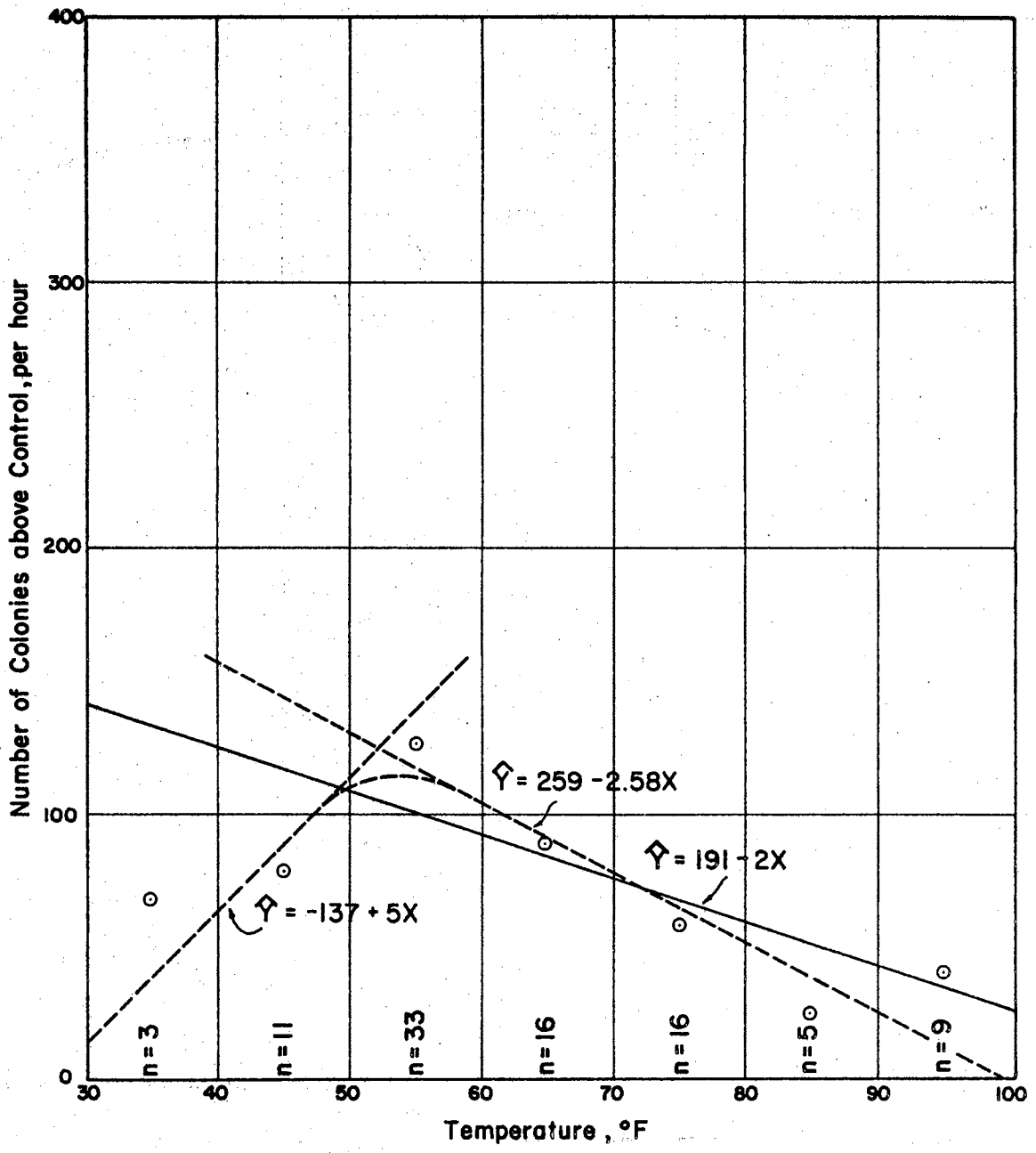

Figure 28 - Relationship between bacterial colonies and temperature - trickling filter.

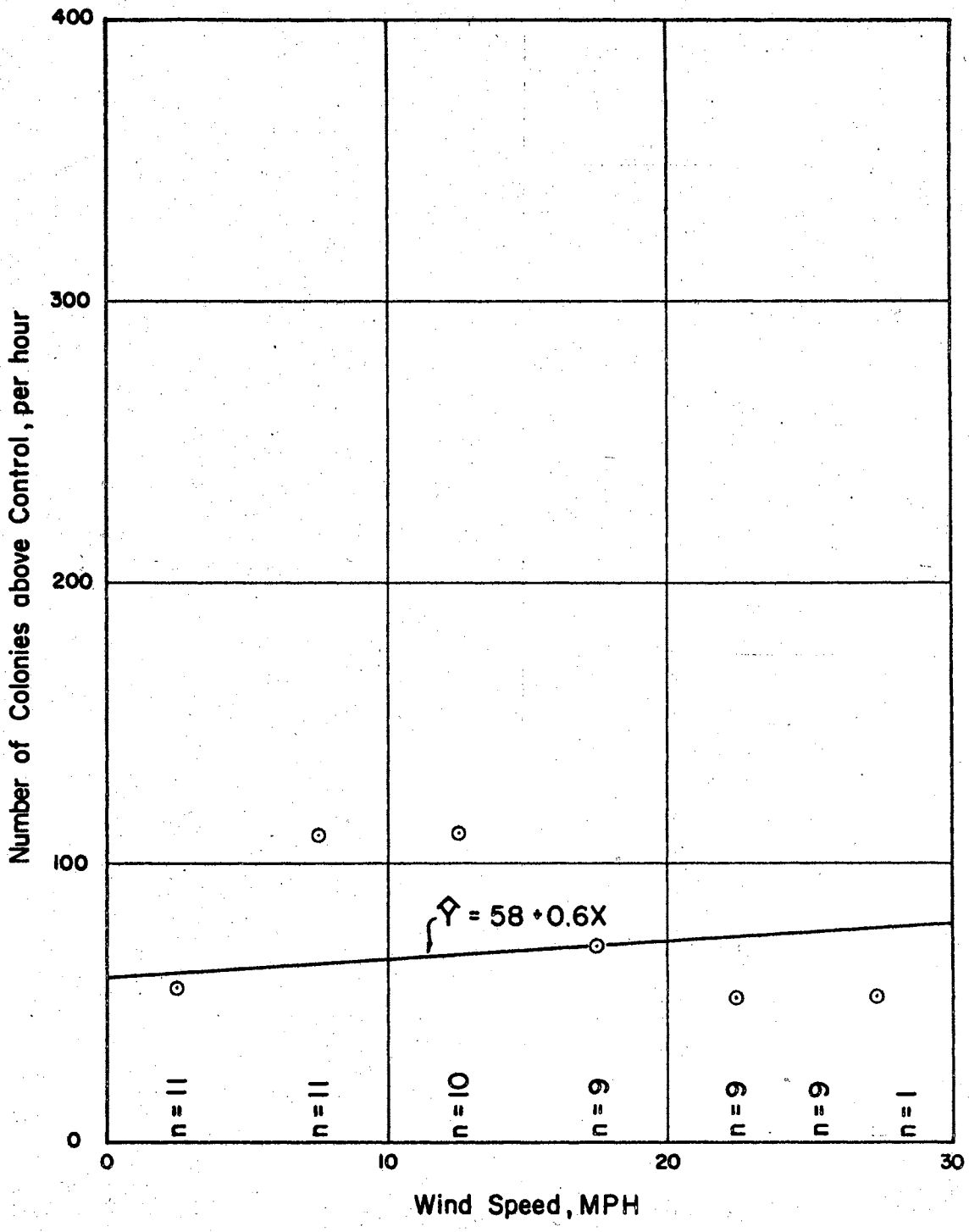

Figure 29 - Relationship between bacterial colonies and wind speed - trickling filter.

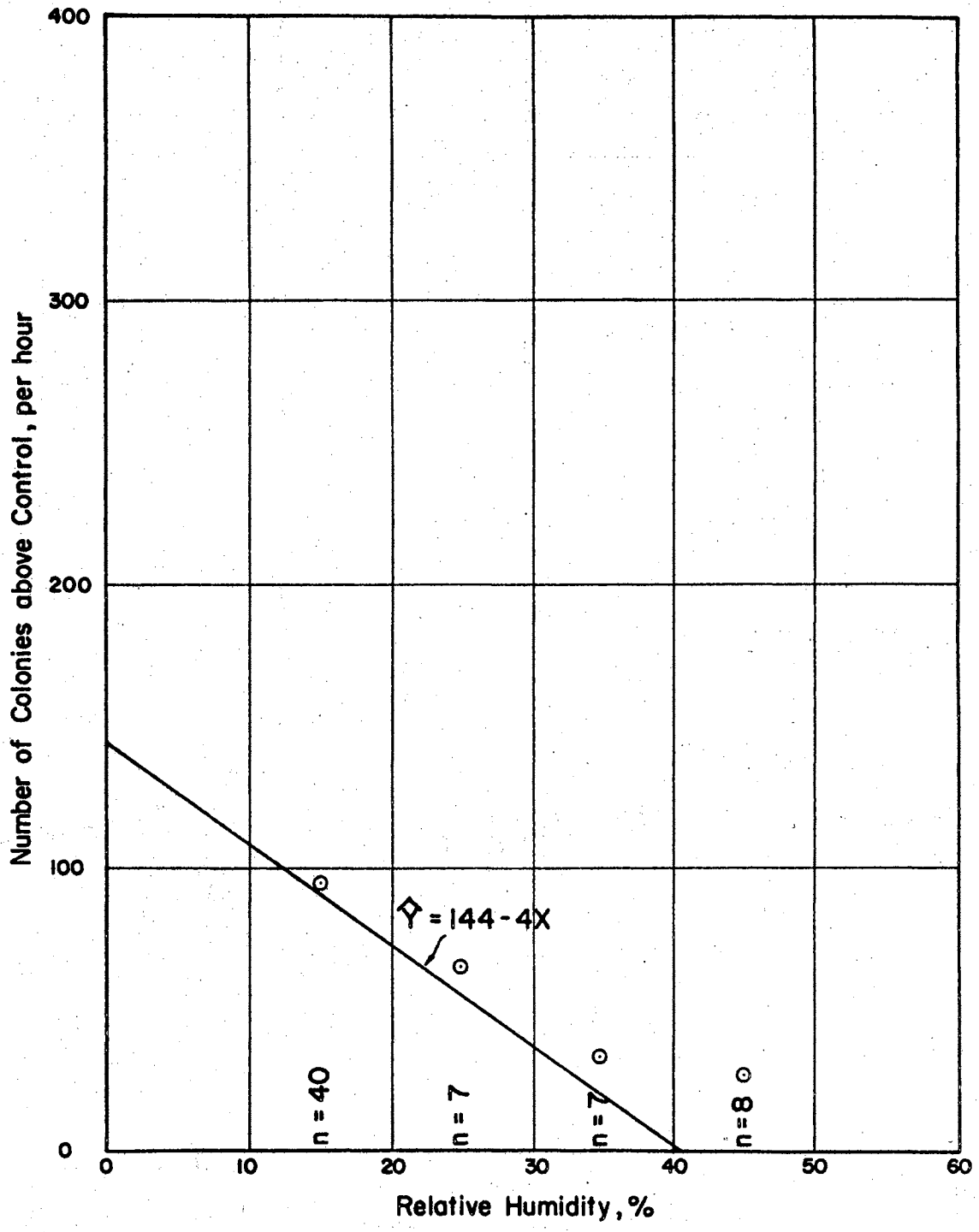

Figure 30 - Relationship between bacterial colonies and relative humidity - trickling filter.

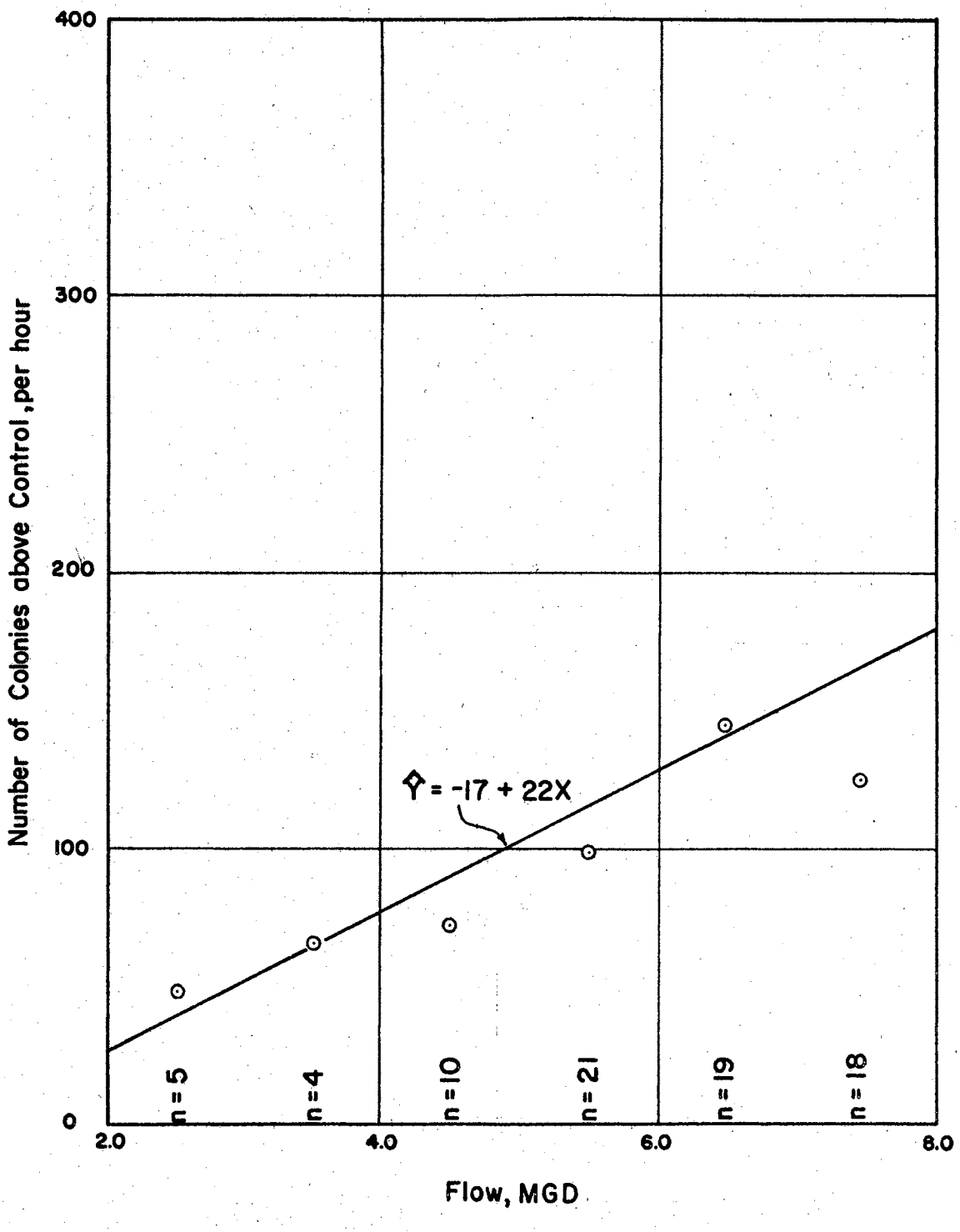

Figure 31 - Relationship between bacterial colonies and flow - activated sludge unit.

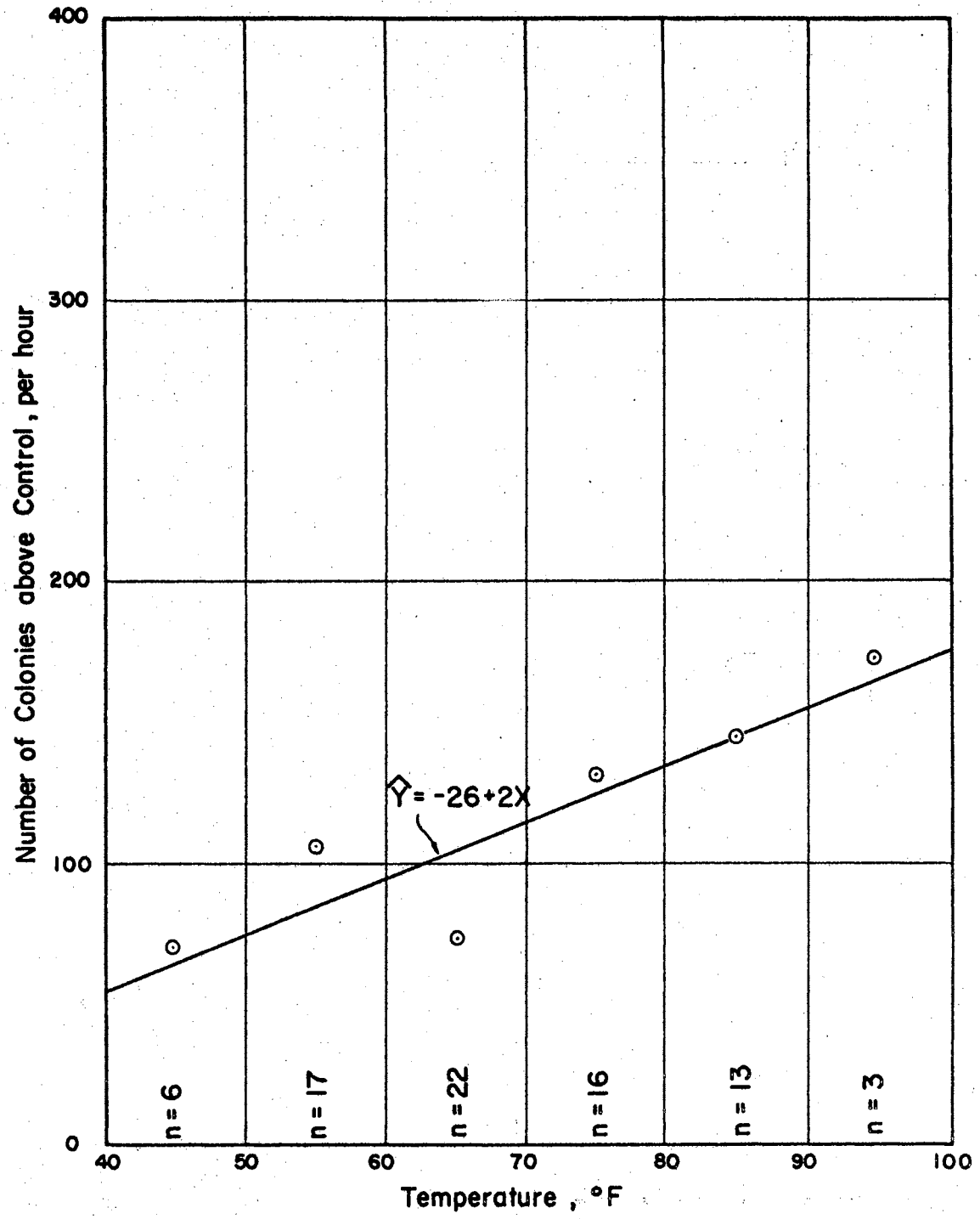

Figure 32 - Relationship between bacterial colonies and temperature - activated sludge unit.

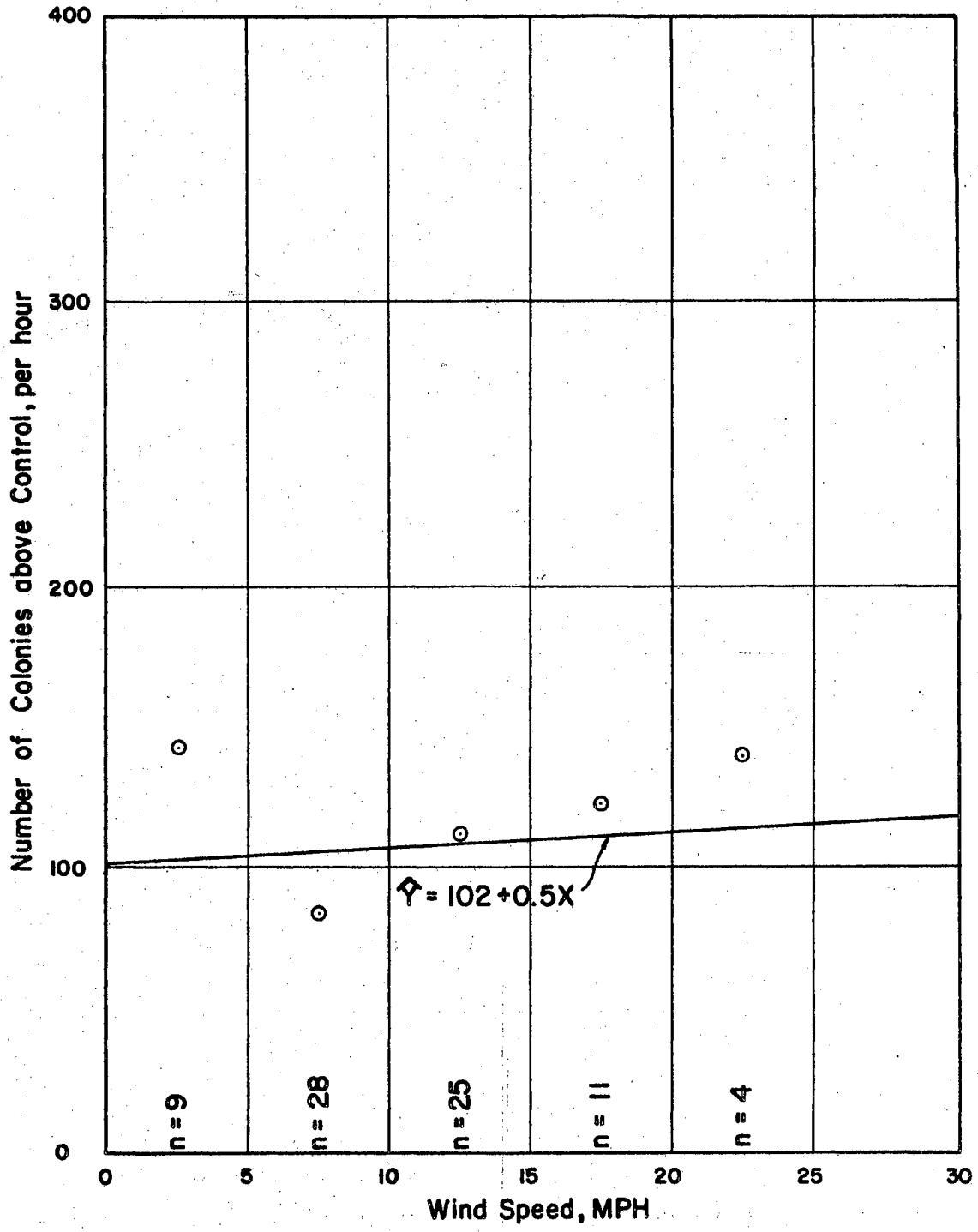

Figure 33 - Relationship between bacterial colonies and wind speed - activated sludge unit.

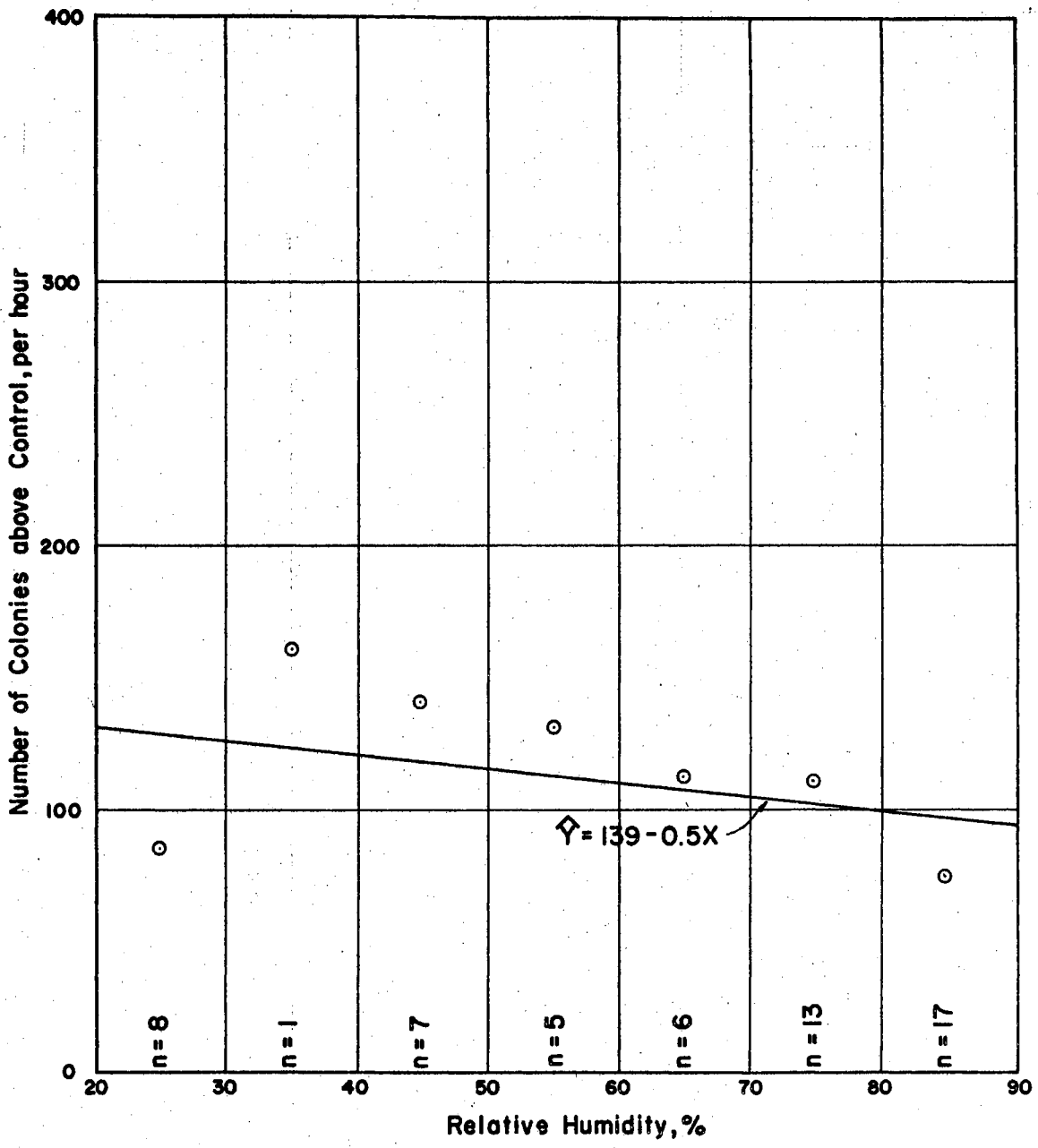

Figure 34 - Relationship between bacterial colonies and relative humidity - activated sludge unit.

indicating a decrease in the number of bacteria emitted with an increase in temperature. The slope of the linear regression line in Figure 32 was positive, showing an increase in the number of colonies emitted as the temperature increased. The number of colonies emitted should increase as the influent temperature increased to some optimum growth temperature. The number of colonies in the air would be expected to decrease with distance, due to the drying effect of the temperature. Figures 25, 29, and 33 show the relationship between the number of colonies emitted and the wind speed. The slope of the linear regression line for the pre-aeration tank was negative, indicating a decrease in bacterial emission as the wind speed increased. The slopes for the trickling filter and activated sludge unit indicated a small increase with wind speed. The relationship between the number of bacteria in the air and wind speed would show a greater effect with distance, if the emission of bacteria were completely dependent upon the wind blowing across a body of water, but the pre-aeration tank and activated sludge unit were forcing bacteria into the atmosphere by air being diffused through the influent while the trickling filter was emitting bacteria by splashing influent over the rock filter. Figures 26, 30, and 34 show the relationship between the bacterial colonies and the relative humidity. The slopes for the linear regression lines show a decrease in the number of colonies with an increase in relative humidity. Relative humidity and tem-

perature are related parameters, and when the two curves are compared they are reversed; that is, when the temperature is high, the relative humidity is low, and when the temperature is low, the relative humidity is high. Because of this comparison, the effects of relative humidity would seem to be reversed with the effect of temperature. This was shown in Figures 32 and 34 for the activated sludge unit. Figures 24, 26, 28, and 32 show the slopes for relative humidity were twice the slopes for temperature. The difference in slopes can be partly attributed to the range of relative humidity tested. Figures 26 and 30 were for relative humidities from ten per cent to forty per cent, and Figure 32 was for forty per cent to one hundred per cent. The plotting of the data showed wide variation that could be explained with multiple degree curves. The product-moment correlation coefficient (r) was calculated for the total temperature and for the dashed lines, curve one, temperatures below 50^{o}F., and curve two, temperatures above 50^{o}F. Figure 24 gave a total value of r equal to -0.407, compared with r_1 equal to 0.182, and r_2 equal to -0.400. These values indicate the linear regression line over the total range was a better fit than breaking the curve into two groups. Figure 28 gave a total r value equal to -0.350, compared with r_1 equal to 0.418, and r_2 equal to -0.456. These values indicate the second degree curve was a better fit for the data than the total linear regression curve. The relationship between colonies

emitted and the parameters indicate that bacterial emission rates were affected by sewage flow, temperature, wind speed, and relative humidity.

Classification

There was no attempt made to identify the types of bacteria; however, certain screening tests were performed. Twenty colonies were collected from each of nine drum samples. Streak-plates were made of the 180 colonies to determine if the bacteria were viable. This test was used to indicate if the bacteria would reproduce if inhaled by the human body. Tryptose agar plates were used to eliminate the shock of a new medium for growth. Colonies from the streak-plates were used as new growth and tryptose blood agar plates were made to check for hemolysis. This test was used to indicate if the bacteria upon entering the lungs would react with the blood cells. Tests for identity were not made, but erosin methyelene blue agar plates were streaked with the new growth, not as a complete presumptive test, but as an indicator for coliform organisms. This test was used because previous work in this area was limited to these organisms. Slides were prepared using the gram stain, and morphology was recorded while determining the staining characteristics. These results are shown in Table IV. The bacterial analysis using the tryptose blood agar plates show 64 beta hemolysis. These colonies would react with human blood and lyse red blood cells. There were fifty-three reactions with eosin methyelene blue agar that indi-

cate a type of coliform organism. Figures 35, 36, and 37 show the bacteria examined with the aid of microscopic photographs. The results of these tests indicate that bacteria emitted from these units could be harmful to man.

TABLE IV

BACTERIAL ANALYSIS

Test	Type	Pre-aeration Tank	Trickling Filter	Activated Sludge
Blood	Alpha	34	44	38
	Beta	26	16	22
	Purple or Other	44	43	40
EMB	Orange, Pink or Other	16	17	20
Gram Stain	Positive	30	36	*33
	Negative	30	24	*7
Morphology	Cocci	20	19	*11
	Bacillus	36	32	*23
	Filamentous	4	9	*6

*Morphology and stain were determined for forty colonies from the activated sludge unit.

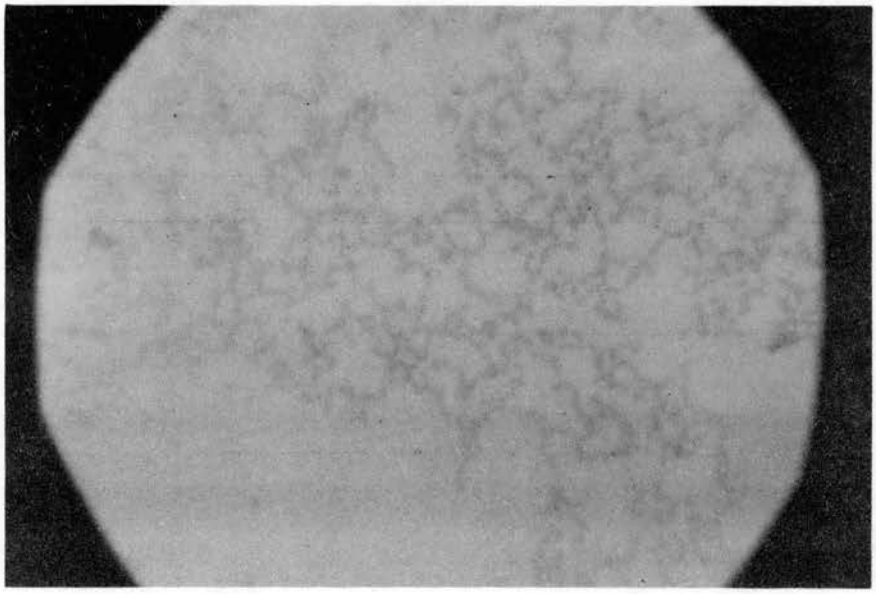

Figure 35 - Bacillus, gram negative.

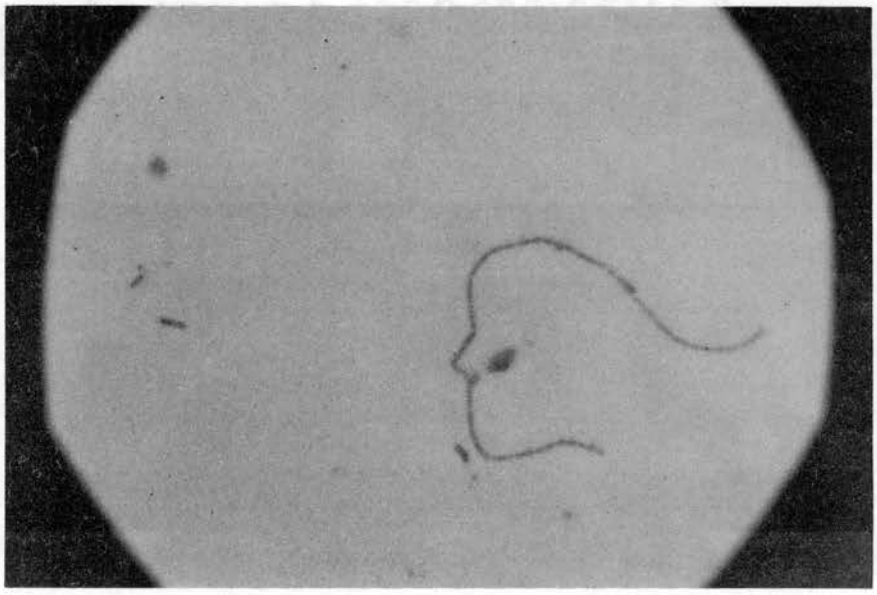

Figure 36 - Bacillus chain, gram negative.

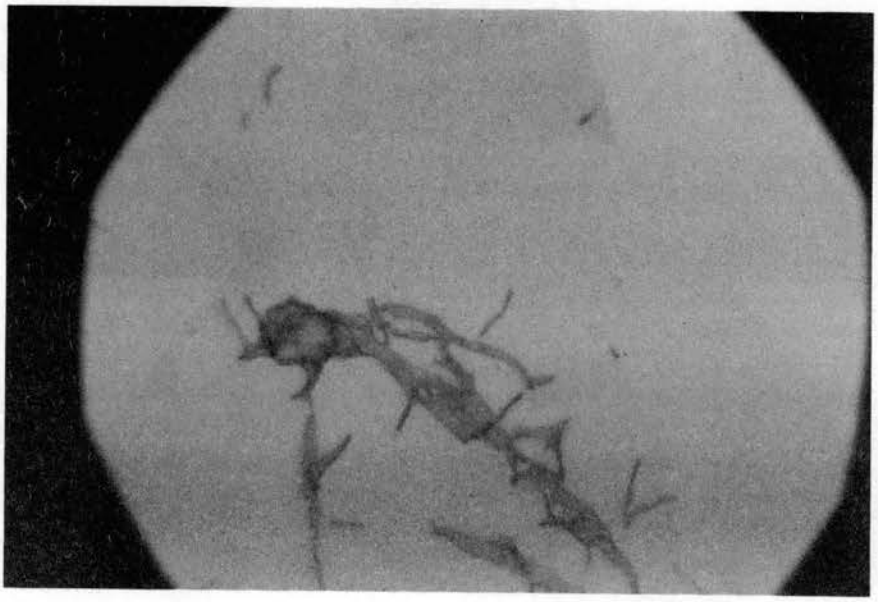

Figure 37 - Filamentous, gram positive.

Tracer Analysis

The final test was conducted using the sieve samplers located at the pre-aeration tank and control one. Hourly samples were collected for ten-minute periods. A tracer bacteria, Bacillus subtilis var. globgii, was obtained from the University of Minnesota. This bacterial strain was used because of its brown pigment, which would allow visual identification on the petri dishes after incubation. The bacteria were inoculated into nine liters of nutrient broth and aerated for forty-eight hours. The nutrient broth was then divided into two equal volumes. The first volume of broth was poured into the drain at the laboratory during the first hour of the test. The laboratory was located approximately five miles from the plant. The second volume was poured into the influent during the seventh hour of sampling at the lift station located approximately one-quarter of a mile from the pre-aeration tank. The colonies were counted after incubation, as shown in Table V.

TABLE V

OBSERVATIONS OF TRACER BACTERIA

	Hour of Test									
	1	2	3	4	5	6	7	8	9	10
number of colonies	64	9	2	4	15	1	1	5	7	8

The high number of colonies obtained during the first hour cannot be explained except by contamination after the test was conducted. The second peak was reached at the

fifth hour, and the number of colonies decreased through the seventh hour when the second volume of broth was poured into the influent. The number of colonies increased through the tenth hour. There were no colonies on the plates collected after the eleven-hour test, and no colonies were observed on control one. These results indicate that a biological tracer could be used and with sufficient data, information gained about threshold limit and viability of the bacteria with distances.

CONCLUSIONS

Bacteria were being emitted from the pre-aeration tank, trickling filter, and activated sludge unit. The drum sampler gave the best representative sample, because it tested continuously for long periods of time through changing independent variables. The results from the drum sampler indicated eight times as much bacteria emitted from the pre-aeration tank, two times from the trickling filter, and two times from the activated sludge unit compared with the normal bacterial load as measured by the controls. The results from the activated sludge unit indicate a lower ratio than the pre-aeration tank, but during the test periods the activated sludge unit was partially covered with foam. This would reduce the airborne bacterial load from this unit, and under other conditions the activated sludge unit, like the pre-aeration tank, could be expected to be higher than the trickling filter. The parameters observed in this research do affect the airborne bacteria. The data

shown here indicate that other parameters might be influencing the bacterial emission rate. Second degree curves could fit the data and explain part of the wide dispersion. The classification of the bacteria indicated that the bacteria emitted from these units would lyse the human blood, and could be harmful if inhaled into the human body.

It was concluded that harmful bacteria were being emitted from wastewater treatment plants, and could be harmful to plant operators and others living close to these areas.

CHAPTER VI

SUMMARY

This research was undertaken to determine if airborne bacteria were being emitted from wastewater treatment units such as aeration tanks and trickling filters. Three samplers were used for the collection of a representative sample: the sequential sampler using membrane filters, the six-stage sieve sampler using tryptose agar as the collection medium, and the drum sampler collecting on tryptose agar for long periods of time. The samples were incubated for twenty-four hours at 37°C. The number of colonies varied from eight times as many bacteria emitted from the pre-aeration tank, two times from the trickling filter, and two times from the activated sludge unit, compared with the normal bacterial load as measured by the controls. The parameters of sewage flow, ambient temperature, wind speed, and relative humidity affect the rate of emission. There was a wide dispersion which indicated other parameters were influencing bacterial emission. Bacteria were not identified as to type, but basic reactions indicated that the bacteria emitted could be harmful if inhaled. The results from the bacterial tracer analysis indicated that a known

bacteria could be added to the influent and collected in the air downwind of the pre-aeration tank.

CHAPTER VII

FUTURE WORK

The results of this research indicated that harmful bacteria were being emitted, and further tests were needed, such as:

1. A study is needed to determine the amount of bacteria emitted from treatment units under various parametric conditions.

2. The effects of distance and bacterial emission should be studied. This might be helped with the aid of a tracer either bacterial, chemical, or radiological.

3. Since viruses have become of greater concern, a study using the sieve sampler and sloppy agar should be made to see if plaques can be detected.

4. The new sampler using liquid medium and thermal plates should be considered for testing bacteria with the aid of membrane filters.

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